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ORIGINAL PAPER

# Kinetic of biogenic sulfide production for microbial consortia isolated from soils with different bioaccessible concentrations of lead

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**Abstract** Different technologies have been implemented for the treatment of acid mine drainage. Among these are technologies such as geochemical barriers and sulfidogenic reactors, which use biogenic sulfide (produced by sulfate-reducing bacteria) for metallic stabilization. Because both processes involve microorganisms, it is important to have a clear understanding of the factors that influence their activity in different toxic environments. Given that microbial communities isolated from polluted sites could have a higher tolerance to toxic ions, two consortia with sulfate-reducing activity were isolated from different soils impacted by mining activities. These soils had different total (401 and 19,300 mg Pb $kg^{-1}$), mobile (54 and 1,415 mg Pb $kg^{-1}$) and bioaccessible (316 and 3,175 mg Pb $kg^{-1}$) concentrations of lead. The kinetics of biogenic sulfide production (BSP) for both consortia were monitored in a batch reactor after they were exposed to different initial lead concentrations. These lead concentrations were established based on the results of lead mobility tests. The estimated BSP rates and biomass concentrations of both consortia showed different responses to the presence of lead. Results highlighted that lead sulfide precipitation on microbial cell is a tolerance mechanism identified and this one is triggered for the lead bioaccessible concentrations threshold in soil. These results could be useful for the designing of processes based on sulfate reducing activity for the removal or stabilization of metal present in water or soil, respectively.

**Keywords** Bioaccessibility · Biogenic sulfide · Kinetic parameters · Soil contamination · Toxicity · Sulfate-reducing bacteria

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## Introduction

Operations that involve mining, processing or smelting of sulfide ores are potential sources of environmental pollution. In the case of the smelting industry, emissions containing a high content of metallic oxides and non-metallic oxides are produced and are sometimes responsible for the contamination of soils and shallow water (Ettler et al. 2007). Additionally, abandoned slag piles located around old smelters contribute to metal dispersion, especially if they are exposed to weathering. Abandoned mine tailing impoundments, which can also be exposed to weathering processes and can release potentially toxic elements into the environment, are another source of contamination (Razo et al. 2004; Blowes et al. 2005; Armienta et al. 2007). The latter are particularly important because they may contain metal sulfides, such as pyrite ($FeS_2$), that produce acid mine drainage (AMD) as a result of the weathering process.

AMD is characterized by elevated concentrations of metals (e.g., 2.1 mg Pb $kg^{-1}$; 13,000 mg FeT $kg^{-1}$; 2,600 mg Zn $kg^{-1}$; 19 mg Cd $kg^{-1}$; 59 mg As $kg^{-1}$) and sulfates (>2,000 mg $kg^{-1}$) and low pH (<3). The oxidation reaction of pyrite has been reported to be (Blowes et al. 2005)

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2H^+ + 2SO_4^{2-} \quad (1)$$

Further oxidation of ferrous iron to ferric iron occurs when oxygen is dissolved in water or when water is exposed to atmospheric oxygen (Eq. 2).

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (2)$$

If the pH > 3, hydrolysis of ferric iron can generate $Fe(OH)_3$, a red–orange precipitate that is found in waters that are affected by AMD. Ferric iron can also react directly with pyrite to produce more ferrous iron and acidity, as shown by Eqs. 3 and 4.

$$2Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3 + 6H^+ \quad (3)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15\,Fe^{2+} + 16H^+ + 2SO_4^{2-} \quad (4)$$

Once generated, AMD can be transported to the soils and percolate through them, thus polluting shallow water and groundwater. Several authors have proposed the use of technologies that involve sulfate-reducing processes to control AMD and the leaching of metals. Among these technologies are sulfidogenic reactors, natural attenuation, and geochemical barriers, such as anaerobic wetlands, successive alkalinity-producing systems and biological barriers (Waybrant et al. 1998; Blowes et al. 2000; Chuichulcherm et al. 2001; Lloyd et al. 2004; Johnson and Hallberg 2005; Church et al. 2007). Sulfate reduction is carried out by bacteria (typically gram-negative bacteria) that are known as sulfate-reducing bacteria (SRB). These bacteria use sulfate ions as the final electron acceptor and short-chain organic compounds (such as volatile fatty acids, VFA) as electron donors. These electron donors can either be completely oxidized to $CO_2$ or partially oxidized to acetic acid, depending on the SRB strain. In the presence of a lactate ion, the simplified reaction of the complete mineralizing process is (Benner et al. 1999)

$$2CH_3CHOHCOO^- + 3SO_4^{2-} + 2H^+ \rightarrow 6HCO_3^- + 3H_2S \quad (5)$$

In the case of incomplete oxidation of the lactate ion, the simplified reaction is expressed as (6).

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + H_2S \quad (6)$$

As can be observed, the sulfate reduction generates alkalinity ($HCO_3^-$) and produces hydrogen sulfide ($H_2S$), which can be used to precipitate metal ions, such as $Me^{2+}$ (Eq. 7) (Parry and Jong 2004)

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + H_2S \quad (7)$$

Therefore, to determine an adequate residence time for the optimal design of either the barriers or reactors, it is important to establish the biogenic sulfide production (BSP) rate and the maximum sulfide concentration production. The BSP rates and the production of biomass depend on several biological and physicochemical factors, including temperature, redox conditions, pH, nutrient concentration, type of organic substrate, and toxic compounds present in the culture media. The performance of SRB during AMD treatment can be affected by the presence of dissolved metals, which may inhibit their normal cellular activity. Furthermore, high metal concentrations may be not only toxic but also lethal to the bacteria. For technologies such as geochemical barriers, it is far more important to have bacteria with a high tolerance for toxic metals than to have a high BSP rate; conversely, in sulfidogenic bioreactors in which the bacteria are protected from direct exposure to toxic metals, it is preferable to have sulfate reducers with a high BSP rate. For this reason, it is necessary to evaluate the effect of different concentrations of toxic elements, such as Pb, on the metabolic behavior of SRB. Cabrera et al. (2006) reported that, when exposed to different concentrations of Cr(III), Cu (II), Mn(II), Ni (II) and Zn (II), *Desulfovibrio* sp. is more tolerant than *Desulfovibrio vulgaris*. Utgikar et al. (2002) reported on an inhibition mechanism of SRB in which metallic sulfide precipitates and covers the cellular membrane. This cover physically interferes with the transport of organic substrates into the cell. Although this effect has only been reported for Cu (II) and Zn (II), it may also occur with other metals. Bharathi et al. (1990) evaluated the toxicity of Pb(II) on a *Desulfosarcina* strain. They found that a low concentration of lead (<100 mg $L^{-1}$) favored the growth of *Desulfosarcina*, a SRB strain. Sani et al. (2003) observed that Pb(II) concentrations ranging from 3 to 15 μM enhanced the lag phase of cell growth. In the aforementioned studies, the inhibition toxicities were analyzed as a function of biomass, sulfate consumption, percentage of metals removed, and duration of the lag phase; however, the effect of Pb(II) concentration on BSP has not been evaluated. Thus, the present study evaluates the kinetics of inhibition toxicity at different Pb(II)

concentrations for two different consortia of bacteria isolated from metal-polluted soils that have distinct sulfate-reducing activities. These consortia were isolated from two sites that have been impacted by mining activities. Lead concentrations to which the sulfate-reducing consortia had been exposed were defined based on the amount of bioaccessible lead, which was measured in the soil samples from which the consortia were isolated. Using a series of microcosm experiments, lag time, biomass production, and the kinetic parameters of BSP were analyzed.

## Materials and methods

### Soil sampling

Soil samples from two areas that had been impacted by mining activities were collected. One of the samples (S1) was collected from soil near slag deposits generated by an old smelter that is no longer in operation in Matehuala-San Luis Potosí. Adjacent to the historical slag piles is a shooting range, which could have also been a source of metallic lead; the longitude and latitude coordinates for the site are 100°38′24″W and 23°39′384″N.

Another sample (S2) was obtained from the Santa Maria de La Paz mining district, which is located at an altitude of 1,858 m above sea level in the north-central part of San Luis Potosí, Mexico (Castillo and Carranza 1996). The longitude and latitude coordinates for this site are 100°42′51″W and 23°40′27″N.

Both soil samples from these sites were taken at a depth of 40 cm from the surface and were stored in nylon bags containing paper sachets (GasPak$^{TM}$ EZ), which helped to generate an anoxic environment during the transportation until the laboratory. In the laboratory, two soil samples were dried at 40 °C for 72 h and subsequently dry soils were sieved to obtain particles of less than 600 microns to be used in total acid digestion, bioaccessibility, and mobility tests. The main sulfides identified as ores in these sites were pyrite, arsenopyrite, galena, sphalerite, chalcopyrite, and bornite (Castillo and Carranza 1996).

One of the aims of this study was to demonstrate that microbial consortia with sulfate-reducing activity in soils with high total concentrations of lead may manifest tolerance mechanisms different from that observed by those consortia from soils with lower concentrations of lead. Successful identification of sulfate-reducing activity in the soil is mainly associated with a deeper sample. The sampling site S1, classified in this study as low total lead concentration soil, corresponds exclusively to a horizon A, and it is found in an area of alluvium deposit where there is accumulation of material from higher zones with evident drag of historical and recent mining wastes. The depth of horizon A of site S1 was approximately 120 cm, but the sample was taken only from the first 40 cm. This is because the site sample S2, which is found in the upper part of a pile of historical tailings, presented a high compaction and only permitted sampling from the first 40 cm. Site S2 was selected because of the high total lead concentration quantified, which corresponds to almost 50 times more that site S1.

### Microorganisms and culture medium

Anaerobic microbial consortia (C1 and C2) were isolated from the samples by placing one gram of soil into a test tube containing Postgate liquid medium (PLM), which contained the following (mg $L^{-1}$): $NH_4Cl$ (1,000), $K_2HPO_4$ (500), $Na_2SO_4$ (4500), $CaCl_2{\cdot}2H_2O$ (40), $MgSO_4{\cdot}7H_2O$ (60), $FeSO_4{\cdot}7H_2O$ (4), sodium thioglycolate (100), ascorbic acid (100), sodium pyruvate (1,100), and sodium lactate (3,000). Agar (10 g $L^{-1}$) was added to create a solid medium. After 1 month of incubation, the tubes took on a dark color, which is indicative of the presence of iron sulfides (Sigalevich et al. 2000). One-milliliter aliquots were spread onto Petri dishes containing Postgate solid medium (PSM) and incubated for 1 month inside an anaerobic chamber. The resulting microbial colonies that showed a dark coloration were isolated and again inoculated in PLM, this time in 125-mL serum bottles that were sealed with neoprene stoppers and aluminum crimps. All of the media were adjusted to a pH of 7 using 0.1 M HCl or NaOH and a Thermo Orion 420A+ pH meter. The media were sterilized by autoclaving for two 15-min cycles at 120 °C and 15 psig and were used for the incubation of the consortia at 30 °C. After 1 month of incubation, the C1 and C2 consortia growing in these systems were utilized in the BSP kinetic tests.

### Total, bioaccessible, and mobile lead concentration in soils

Different extraction procedures were used to determine the total, bioaccessible, and mobile lead concentrations in the soil samples. Total lead concentrations were determined using the ASTM D 5258-92 method modified. This method consisted in an acid total digestion of dry soil (0.5 g) using PTFE-insets of microwave

digestion vessels. Microwave-assisted heavy metal extraction was carried out with 25 mL of a mixture of 25 % HNO3—10 % HCl in a CEM Mars X unit at 350 psi and 170 °C for 15 min. The solution was brought to 50 mL in a glass flask and filtered through paper filters into polyethylene bottles. A standard reference soil (NIST 2710, a Montana soil sample with high concentrations of trace elements) was employed for an analytical quality control. Lead bioaccessibility tests were carried out following the simple bioaccessibility extraction test developed by the Solubility/Bioavailability Research Consortium (Omen et al. 2002). Because the SRB could be exposed to organic acids they themselves produced (e.g., acetic acid), mobility tests were carried out following the 1311 USEPA method. This method utilizes 0.1 M acetic acid solution as an extractant, with a solid/extractant ratio of 1:20. Moreover, because acetic acid solution could be produced in situ by incomplete oxidization (Gottschalk 1986) the solid/extractant ratio was increased to 1:200.

### Kinetic studies of two anaerobic lead tolerant microbial consortia

Biogenic hydrogen sulfide production, in both the absence and presence of lead, was evaluated using the C1 and C2 microbial consortia. All of the tests were carried out in duplicate in 120-mL serum bottles using 120 mL of PLM and kept at a temperature of 30 °C and orbitally agitated at 100 rpm (Max$^{Q}$ 2000). The total sulfate concentration in the PLM was 1.5 g $L^{-1}$; however, the ratio of each sulfate source varied depending on the ferrous ion concentration that was necessary to precipitate the initial biogenic sulfide from the culture aliquots that were used as inoculums. In this way, the initial biogenic sulfide was removed prior to kinetic measurement in all of the tests. In the case of the C1 microbial consortium, sulfate was added in the following forms: $Na_2SO_4$ (2,183 mg $L^{-1}$), $MgSO_4{\cdot}7H_2O$ (60 mg $L^{-1}$), and $FeSO_4{\cdot}7H_2O$ (12 mg $L^{-1}$). In the case of the C2 microbial consortium, sulfate was added in the following forms: $NaSO_4$ (2,104 mg $L^{-1}$), $MgSO_4{\cdot}7H_2O$ (28 mg $L^{-1}$), and $FeSO_4{\cdot}7H_2O$ (154 mg $L^{-1}$). Sodium lactate was used as the only electron donor in the kinetic tests, and it was added at two time points to achieve a concentration of 0.93 g $L^{-1}$ each time. The first sodium lactate addition was made at the beginning of the experiment. The second addition was made after 70 h to obtain a more definitive measurement of the growth inhibition effect of lead, as suggested by Angelidaki et al. (2007). Lead was added to serum bottles in the form of lead nitrate, $Pb(NO_3)_2$, and the lead concentration range that was used was based on the results of the soil mobility tests. Thus, it was established that the minimum and maximum mobile lead concentrations were 34 and 3,380 μM, respectively. A culture without lead was used for a control test. Finally, endogenous respiration was taken into account by means of tests without the addition of metal and sodium lactate. The residual inorganic precipitates were analyzed by scanning electron microscopy coupled to emission disperse spectroscopy (SEM–EDS).

### Analytical methods

The aqueous solutions obtained from different extraction procedures were analyzed by absorption atomic spectrometry with a spectrometer (Varian Spectra AA 220). The biogenic sulfide content was determined through a turbidimetric method developed by Cord-Ruwisch (1985) using a spectrophotometer UV–Vis (Beckman, D.U. 650). The kinetic parameters, including the first-order kinetic constant, the maximum biogenic hydrogen sulfide concentration, the lag phase duration and the maximal specific rate of BSP, were determined using the Gompertz sigmoidal model, which has been employed in several studies on phenanthrene biodegradation in bioreactors, the growth of microorganisms, and the biodegradation of several chemical compounds (Lay et al. 1998; Ortiz et al. 2003).

The biomass in the bottles was evaluated when the BSP rate reached steady state and was quantified as the amount of volatile solids suspended (VSS) according to method 2540 E (Eaton et al. 2005) employing a muffle furnace (Vulcan$^{TM}$ 3-550PD). The half-maximal inhibitory concentration ($IC_{50}$) of lead in cell growth was determined using a dose–response logistic model that has been widely used in toxicological, biological, and pharmacological studies (Eggenschwiler et al. 2007; Beckon et al. 2008).

The biogenic precipitates formed during kinetic tests in the presence of lead were recuperated after VSS determination of their mineralogical characteristics (morphological and chemical) using scanning electron microscopy coupled to energy-dispersive spectroscopy (SEM–EDS). The solids were mounted on aluminum pins, which were covered with gold to minimize mineral alteration and to create conductive surfaces. The characterization was performed using a SEM Phillips XL 30 microscope equipped with a 30-mm$^2$ energy-dispersive Si(Li) detector EDAX DX4 for chemical analysis; secondary and backscattered electron detectors were used for imaging. An electron energy of 20 keV and a beam current of 50 nA were employed for the microanalysis.

**Table 1** Total, bioaccessible, and mobile concentration of lead in the S1 and S2 soil samples

| Sample | Total | Bioaccessible | Mobile (a) | Mobile (b) |
|---|---|---|---|---|
| S1 | 401 ± 20 | 316 ± 14 | ND | ND |
| S2 | 19,300 ± 4,600 | 3,175 ± 45 | 54 ± 1.1 | 1,415 ± 267 |

All determinations were realized by duplicate. All concentrations are in mg Pb $kg^{-1}$ soil. Mobile test carried out with a solid/extractant ratio of (a) 1:20 and (b) 1:200

*ND* no detectable

## Results and discussion

### Physicochemical lead analysis

The results of the physicochemical characterization of both soil samples are presented in Table 1. A very high total lead concentration, 19,300 mg Pb $kg^{-1}$, was found in the soil sample collected from the top of the historic tailings impoundment (S2). Conversely, sample S1 had a total lead concentration of 401 mg Pb $kg^{-1}$. In both cases, the total lead concentrations were above the world average concentration (25 mg Pb $kg^{-1}$) established for superficial soils (Kabata-Pendias and Pendias 2001); however, only in the case of S2 was the lead concentration considered to be above the level set in the Mexican regulations for agricultural/residential or industrial soils (DOF Official Journal of the Federation 2007). The recuperation obtained from the NIST 2710 sample in the total lead concentration analysis was 80 %.

The bioaccessibility results show that the S2 soil sample has a bioaccessible concentration of 3,175 mg Pb $kg^{-1}$, which is one order of magnitude higher than that of the S1 soil sample (316 mg Pb $kg^{-1}$); this difference indicates that the consortium isolated from S2 could have a higher tolerance to lead. Diaz Raviña and Bååth (1996) and Gadd (2010) have previously reported that bacteria isolated from soils that are artificially polluted with metals have a higher tolerance to toxic metals.

In the case of S2, the bioaccessible lead concentration could be related to sulfide weathering and, consequently, the presence of secondary phases (e.g., lead oxides, cerussite, lead hydroxide, and anglesite) with higher solubilities than those of sulfide minerals (Ruby et al. 1999). In the case of S1, the bioaccessible concentration represents approximately 79 % of the total lead concentration; this result indicates that most of the lead is associated with lead oxide phases, which likely originated from the nearby historic smelter sites rather than the shooting range (Ruby et al. 1999).

Finally, Table 1 shows the total and bioaccessible lead concentrations obtained for both samples (S1 and S2). The mobile concentrations in both soils samples were measured using two solid/extractant ratios to establish the minimum and maximum limits for lead exposure. The mobile lead concentration was only detectable for the sample with higher bioaccessible lead concentration (S2).

In case of S2 sample, the results showed that the lead mobility for a solid/extractant ratio of 1:20 was 54 mg $kg^{-1}$; however, when this ratio was changed to 1:200, the lead concentration reached values of up to 1,415 mg $kg^{-1}$. This increase could be related to a stronger metal–ligand interaction produced by the higher concentration of available carboxylic groups.

### Biogenic sulfide production kinetics

The microbial consortia (C1 and C2) obtained from the soil samples were exposed to the following lead concentrations: 34, 168, 337, 1,687, and 3,380 μM. Based on the study of Angelidaki et al. (2007) sulfide production was measured after the second addition of lactate (70 h from the initiation of the test). BSP due to endogenous respiration was detected in neither C1 nor C2 with the calibration curve employed; hence, it was considered negligible.

Figure 1 shows the results of BSP under different lead concentrations for both consortia. When lead was not added (Fig. 1a), the BSP response of C1 is higher than that of C2. As the concentration of lead is increased, the BSP response in C1 is negatively affected, resulting in a delay in the BSP lag phase and a decrease in the BSP rate (Fig. 1b–f). Conversely, in the case of C2, a negative effect is only observed at concentrations above 1,687 μM; at concentrations below this value, no effect on BSP was observed. A positive effect (a slight increase in BSP) was observed for a lead concentration of 1,687 μM.

To improve the interpretation of these results, a fitting of the data using the Gompertz model was performed. This model obtains the following kinetic parameters: the first-order kinetic constant ($k$), maximum biogenic hydrogen sulfide concentration, lag phase, and the maximal specific rate ($r_{max}$) of BSP (Table 2).

As expected, the lag phase for C2 is lower than that observed for C1, which could be due to a higher tolerance to lead, as highlighted by the bioaccessible concentration results for this consortium. This behavior has been previously observed by Cabrera et al. (2006) and Sani et al. (2003) using other heavy metals.

A good correlation between the first-order kinetic constant and the $r_{max}$ was found for both consortia. When C1 was cultured in absence of lead, the $r_{max}$ reached values of

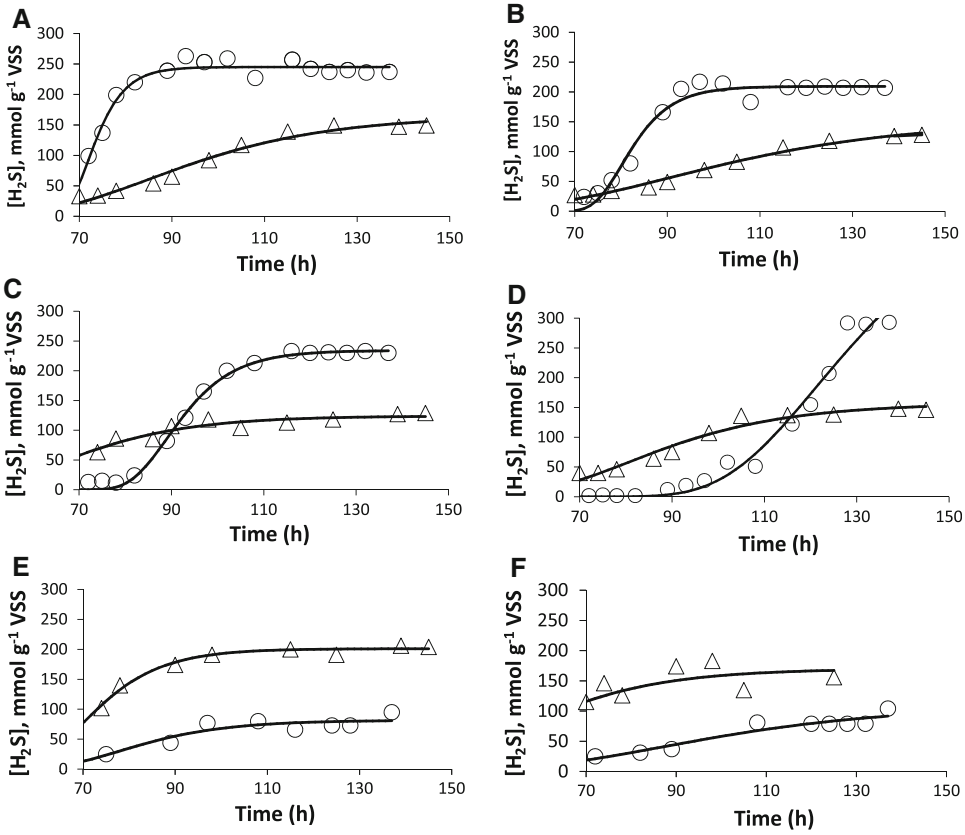


**Fig. 1** Effect of lead concentration on the specific biogenic sulfide produced by the C1 (*open circle*) and C2 (*open triangle*) microbial consortia (**a**) in the absence of Pb, or at different initial lead concentrations (μM): **b** 34; **c** 169; **d** 337; **e** 1,687; and **f** 3,380. The continuous lines represent the Gompertz model fitting

**Table 2** Kinetic parameters for C1 and C2 consortia for each lead concentration determined by means of the Gompertz model fit

| Consortium | [Pb], μM | $a$ | $k$ ($h^{-1}$) | $r_{max}$ mmol $H_2S$ $g^{-1}$ SSV $h^{-1}$ | Lag time (h) | $t_c$ (h) | $R$ |
|---|---|---|---|---|---|---|---|
| C1 | | | | | | | |
| A | 0 | 245.0 | 0.228 | 21.5 | 63.3 | 71.9 | 0.975 |
| B | 34.12 | 209.2 | 0.168 | 13.6 | 69.3 | 80.1 | 0.972 |
| C | 168.9 | 234.4 | 0.126 | 11.4 | 76.3 | 89.0 | 0.995 |
| D | 337 | 504.8 | 0.050 | 9.7 | 82.0 | 121.4 | 0.972 |
| E | 1687 | 81.6 | 0.077 | 2.4 | 58 | 77.9 | 0.874 |
| F | 3378 | 109.7 | 0.034 | 1.4 | 41 | 86.8 | 0.923 |
| C2 | | | | | | | |
| A | 0 | 165.5 | 0.046 | 3.0 | 51 | 85.1 | 0.977 |
| B | 34.12 | 152.2 | 0.035 | 2.1 | 43.6 | 90.5 | 0.985 |
| C | 168.9 | 123.9 | 0.063 | 3.0 | 41 | 65.8 | 0.956 |
| D | 337 | 157.0 | 0.052 | 3.2 | 37 | 80.5 | 0.970 |
| E | 1,687 | 201 | 0.104 | 8.1 | 53 | 69.6 | 0.991 |
| F | 3,378 | 170.0 | 0.058 | 3.8 | 25 | 53.4 | 0.864 |

up to 21.5 mmol $H_2S$ $g^{-1}$ VSS $h^{-1}$. Meanwhile, in the presence of lead, the $r_{max}$ values exponentially decayed as the lead concentration increased (Fig. 2). This is in agreement with the results of toxicological study by Nies (1999) that showed that the interaction of lead ions with sulfhydryl (–SH) groups inhibits enzymatic activity due to competition with toxic heavy metals for ions that are essential for cell activity. However, other authors (Utgikar et al. 2002) proposed that the limitation on the transport of both sulfate and organic substrate towards active sites of

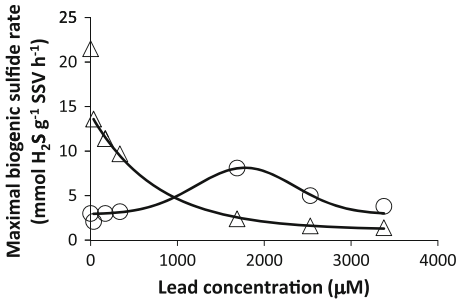


**Fig. 2** Maximal biogenic sulfide production rates as a function of initial lead concentration for the C1 (*open circle*) and C2 (*open triangle*) microbial consortia. The data fitting was performed using an exponential decay of second order for C1 and a Gaussian model for C2

enzymes is caused by the precipitation of lead sulfide that results in the formation of a coating (physical barrier) on the cell surface.

Figure 2 shows that for consortium C2, $r_{max}$ is invariable up to lead concentrations of 337 μM, with a steady value of 2.8 mmol $H_2S$ $h^{-1}$ $g^{-1}$ VSS. Above this concentration, $r_{max}$ rises to values of 8 mmol $H_2S$ $h^{-1}$ $g^{-1}$ VSS, with a sudden decrease at concentrations of 1,687 μM. It is apparent from this behavior that C2 could have a threshold that activates a lead tolerance mechanism that increases the BSP rate. The mechanism for this lead behavior is complex, and some studies have tried to explain it based on molecular biological models (Borremans et al. 2001) and have suggested either extracellular precipitation or intracellular accumulation as mechanisms. It has been proposed that in the latter case, the accumulation occurs as a result of binding interactions of lead with cysteine, which depend on the functional groups (ammine, carboxylic and thiol) that bind to lead (Baker and Czarnecki-Maulden 1987). In the case of extracellular precipitation, scanning electron micrographs have revealed the formation of lead sulfide precipitates.

Figure 3 shows a SEM image of solid particles recovered during biomass determinations from tests with consortium C2 exposed to 1,687 μM of Pb. Considering that backscattered electron mode in scanning electron microscopy the mineral phases containing lead are brighter than others, the EDS spectra were obtained by selecting particles with a higher brightness. The results obtained of EDS analysis showed that the atomic percentages of sulfur and lead have a ratio almost close to 1:1, thus confirms the presence of a solid phase of biogenic lead sulfide.

It is known that hydrogen sulfide has an inhibitory effect on SRB (Postgate 1984; Reis et al. 1992; Moosa and Harrison 2006; Thauer et al. 2007); however, Utgikar et al. (2002) have shown that in the presence of low concentrations of metallic ions, hydrogen sulfide could have a beneficial effect because it helps to remove the ions by precipitation. Bharathi et al. (1990) report higher biomass production in cultures with low lead concentrations compared to those without lead. This increased production is related to the formation of metallic sulfide precipitates, which keeps both toxic species at a minimum level (the ratio of Pb(II) and $H_2S$ is 1:1). In this study, this effect was analyzed using the correlation between a dimensionless parameter and the lead concentrations in solution. The dimensionless parameter ($\theta$) named normalized biomass production (Fig. 4) was calculated as the ratio between the biomass produced in a

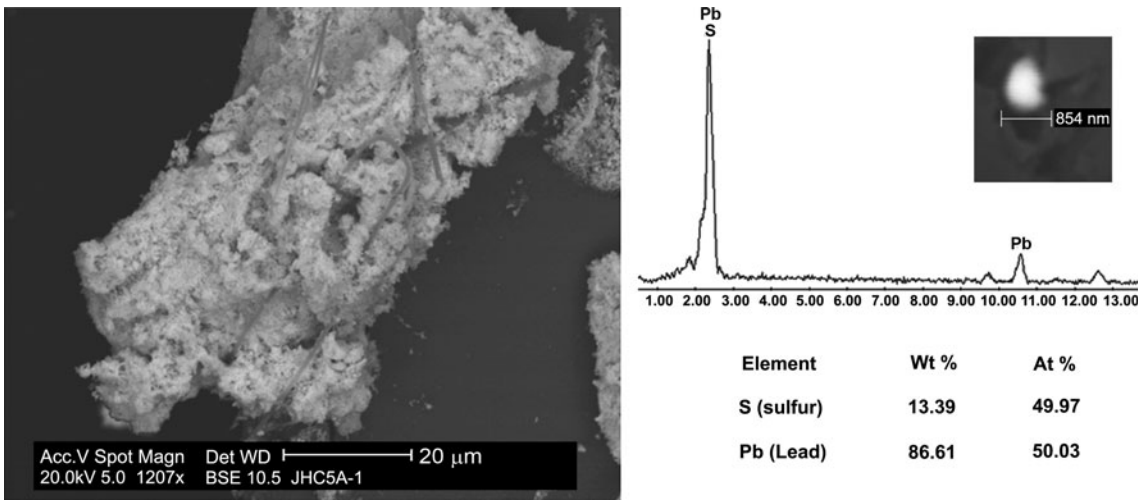


**Fig. 3** Scanning electron micrograph of the solid particles recovered from the fixed solid tests (*left*) and the EDS analyses (*right*) that suggest the presence of lead sulfide

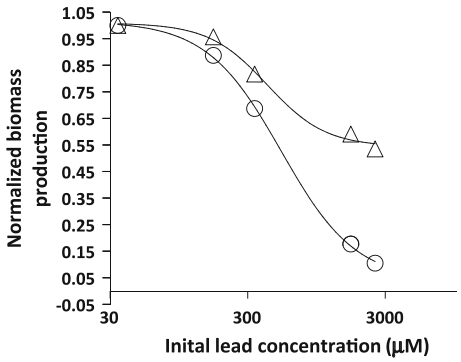


**Fig. 4** Dimensionless normalized biomass parameter for each microbial consortia, C1 (*open circle*) and C2 (*open triangle*). The continuous lines represent the fit to a logistic decay model

**Table 3** Estimation of the half maximal inhibitory concentration of lead on cell growth ($IC_{50}$) for C1 and C2 consortia

| Parameter | C1 | C2 |
|---|---|---|
| $\theta_{max}$ | 1.01 ± 0.007 | 1.01 ± 0.04 |
| $\theta_{min}$ | 0.075 ± 0.01 | 0.52 ± 0.07 |
| $[Pb^{2+}]_{\theta = 0.5}$ | 559 | 2530 |
| $R^2$ | 0.99 | 0.99 |

specific lead concentration and the biomass produced at the most lower lead concentration. The biomass results demonstrate the negative effect of lead concentration, which was more detrimental for the C1 consortium. Interestingly, all of these cultures had a higher biomass production than the cultures without Pb, which only achieved a biomass production of 60 and 93 mg VSS $L^{-1}$ for C1 and C2, respectively. These results confirm that a low concentration of Pb creates an environment that is free of hydrogen sulfide toxicity and thus favoring cellular synthesis.

According to Angelidaki et al. (2007) the determination of the inhibitory effect of specific compounds is generally carried out to establish the $IC_{50}$ value, which represents the concentration that produces a 50 % decrease in the capacity of cellular synthesis or biomass generation. With the purpose of estimating the $IC_{50}$ for Pb of both consortia studied here, the results of Fig. 4 were adjusted to the logistic model of dose–response (Eggenschwiler et al. 2007), represented by

$$\theta = \theta_{max} + \frac{\theta_{min} - \theta_{max}}{1 + ([Pb^{2+}]/[Pb^{2+}]_{\theta=50})^{p}} \quad (8)$$

where $\theta$ represents the normalized biomass, the parameters $\theta_{min}$ and $\theta_{max}$ define the cultures with the highest and lowest biomass concentration, $p$ indicates the slope value at the inflection point of the curve, and $[Pb^{2+}]_{\theta = 0.5}$ represents the $IC_{50}$.

The fit parameters that were evaluated using the logistic model are shown in Table 3. It can be observed that for lead concentrations of approximately 560 µM, the biomass production decreases to 50 % in the $C_1$ consortium. In the case of the C2 consortium, the maximal lead concentration tested in this study causes a 52 % reduction in maximal biomass production. Therefore, exposure to these lead concentrations suggests that the energy obtained by the metabolic processes is distributed mainly for cellular maintenance (catabolic metabolism) and not for biosynthesis (anabolic metabolism).

## Conclusion

The physicochemical results of the soils studied here revealed a high content of lead species derived from mining, milling, and smelting activities. The bioaccessibility results indicated that the S2 soil has a higher concentration of bioaccessible lead (3,175 mg $kg^{-1}$) than the S1 soil (316 mg $kg^{-1}$). The high lead bioaccessibility in the S2 soil could be attributed to sulfide weathering because this soil was formed naturally on top of a historic tailing impoundment. The consortium (C2) isolated from the soil with the highest lead concentration (S2) showed a higher tolerance when exposed to high concentrations of lead in solution. This suggests that microbial communities promote phenotypical responses to toxic exposure.

Kinetic analysis indicated that the consortia behaved differently. The results of the lag phase allow us to conclude that $H_2S$ formation is not an adequate parameter for evaluating the tolerance of the microbial consortia, given that it is consumed by precipitation reactions, such as lead precipitation (PbS). However, the rate of sulfide formation contributed to the correlation observed between tolerance and lead concentration. The results from the SEM suggested that extracellular precipitation is a mechanism for lead tolerance. It was noteworthy that there was a difference in biomass formation based on lead tolerance, as the consortia with lower tolerance to lead reduced its cellular synthesis capacity to as low as 10 %, while the consortia with higher tolerance to lead only reduced its capacity to 50 %, even following exposure to the highest lead concentration.

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