



## Research article

## Developing a biosurfactant to attenuate arsenic contamination in mining tailings

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## ABSTRACT

The present study aimed to investigate the ability of a microbial consortium to produce biosurfactant in the presence of two carbon sources and also to evaluate the efficiency of the cell-free supernatant cultures to mobilize As from naturally contaminated soil. *Pseudomonas* and *Stenotrophomonas* were the main microorganisms in the microbial consortium. The pH, the incubation time, the temperature, and the glucose and glycerol ratios in the culture medium are the main factors influencing biosurfactant production. The lowest surface tension, 30 mN.m<sup>-1</sup>, and the higher emulsification index, 58%, were achieved at the optimum production conditions (OPC), i.e., pH 9.5, a 2.5 glucose/glycerol ratio, after three days of incubation at 25 °C. The cell-free extracts containing biosurfactants were more efficient in mobilizing As than distilled water, CaCl<sub>2</sub> 0.1 mol.L<sup>-1</sup>; saponin, 0.1%; or sodium dodecyl sulfate, 1% during a sequential soil-flushing procedure. The As mobilization using the supernatants containing biosurfactant was sensitive to pH. The use of OPC cell-free supernatant under alkaline conditions leads to the best-obtained results: 24.6% of As removal (678 mg.kg<sup>-1</sup>) during sequential extractions. The toxicity reduction of the column eluted solution from the first to the seventh cycle evaluated by the germination index (GI) and morphological structures of *Allium cepa* in the ecotoxicological assessment confirmed the efficiency of the proposed treatment. *Allium cepa* seeds and seedlings were sensitive in detecting As in soil and eluted solutions with short time responses to the contaminant. Seeds development increased gradually with arsenic removal. The germination index rose from 0 to 55% after soil decontamination through the proposed soil-flushing procedure.

## 1. Introduction

Arsenic is an element widely distributed in the environment by natural and anthropogenic factors and usually found in association with Au, O, or S minerals. The main arsenic-containing minerals are arsenopyrite (FeAsS), löllingite (FeAs<sub>2</sub>), and pyrite (FeS<sub>2</sub>), where As could be present as a trace element. In the environment, As exists in four oxidations states (+5, +3, 0, and -3) and forms diverse organic and inorganic compounds (Ali, 2018; O'Day, 2006; Rashidi Nodeh et al., 2016). Its inorganic species are more mobile and toxic than the organic forms, especially the arsenite, As (III), species considered more harmful than the oxidized As (V) (Smedley and Kinniburgh, 2002).

The environmental impacts of arsenic contamination result from the natural and anthropogenic mobilization of this toxic element. Weathering, erosion, and biological activity on As-rich geological formation are

the primary sources of the natural release of arsenic to the environment (Herath et al., 2016). Arsenic anthropogenic sources include fossil fuel exploitation and combustion, industrial uses of As-enriched materials, agricultural activities, and mining and smelting of As-containing minerals (Wang and Mulligan, 2009a). Historically, the Minas Gerais State in Brazil, particularly the most prominent region, the so-called Iron Quadrangle (IQ), is an area under intense mining activities since the 17th century. The As distribution in the rocks in the region is strongly associated with the sulfidic gold ores. The IQ possesses approximately sixty mines, and, after more than 300 years of exploitation, it is presumably more than 390000 tons of arsenic wastes had been produced and discharged in the whole IQ area (Teixeira et al., 2020; Figueiredo et al., 2007). Within Ouro Preto and Mariana cities' urban perimeter, several underground mine galleries were dug and explored in ancient times.

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Mining tailings exposure to the air and humidity contributes to As release in the environment (Teixeira et al., 2020).

Water is an essential constituent for life in the Earth ecosystem. The growth of population and anthropogenic activities increased the demand and awareness of its importance. Unfortunately, the quality of water resources deteriorates continuously by the addition of undesirable chemicals (Basheer, 2018). Metals, pesticides, herbicides, industrial chemicals, drugs, and their metabolites are found in water and disturb, directly or indirectly, the environment (Alharbi et al., 2018). The solubilized As, for example, could be carried out to the superficial and groundwater water deposits, thus compromising the drinking water quality. Since the toxicity of inorganic As species are recognized, it configures a potential risk to the human population, as observed in China (Rodriguez-Lado et al., 2013), Pakistan (Shahid et al., 2018), India (Chakraborti et al., 2015), and many other countries. Then, in different sites near Ouro Preto, concentrations of As above  $10 \mu\text{g.L}^{-1}$ , the maximum level recommended for drinking water by the World Health Organization (WHO, 2017), are usual (Borba et al., 2004). Ecotoxicology assays are essential tools to assess or predict the environmental impacts of contaminants. In that way, diverse animal and plant species are useful biosensors (van Gestel, 2012). *Solanum lycopersicon*, *Allium cepa*, *Helianthus annuus*, and *Oryza sativa* are plant species frequently used in ecotoxicity testing due to their seedling and growing properties and easily detectable responses to the environment alterations (OECD 312, 2004; OECD, 2006; Quadra et al., 2019). Ecotoxicity assessments are applied to estimate the toxicity of soils, effluents, and water. It is a new and exciting approach for those interested in evaluating the attenuation of some environmental contamination.

The decontamination of As-containing soils could be achieved by liquid extraction procedures using several liquid extractants, including the surfactants. Surfactants are classified as amphipathic compounds since both, hydrophobic and hydrophilic groups, are present in their structures. Microorganisms usually secrete metabolites, including surfactants (so-called biosurfactants), to increase nutrients availability in the growing medium. The production of these bioproducts is sensitive to the growing conditions, mainly the carbon sources, the pH, and the temperature. Biosurfactants are interesting bioproducts due to their excellent physicochemical properties. Those bioproducts tolerate broad temperature and pH ranges, possess emulsifying properties, and are biocompatible given their low toxicity. Therefore, these features turned these products into promising candidates for environmental applications (Sarubbo et al., 2015). The properties mentioned above explain the potential of using these biomolecules for environmentally-friendly remediation processes. These substances may contribute to the solubilization, mobilization, dispersion, and desorption of inorganic or organic contaminants (Mulligan, 2009; Franzetti et al., 2010; Cameotra and Makkar, 2010). The mobilization of metal ions and other soil contaminants by biosurfactants is of interest and had received increased attention recently (Chakraborty and Das, 2014; Franzetti et al., 2014; Sarubbo et al., 2015). There is an abundance of scientific reports concerning biosurfactant production and its environmental applications using isolated microbial strains. Still, the use of microbial consortia with these objectives is scarce. Therefore, the usefulness and the efficiency of mixed cultures with those purposes needs attention and a detailed investigation.

Hence, this study, rather than using pure microbial cultures enriched in a medium containing a single carbon source, aimed to utilize a microbial consortium obtained from pond sediments, to produce a biosurfactant-enriched extract capable of promoting As extraction from contaminated soil through soil-flushing. This research addressed particular attention to the use of a mixture of glucose and glycerol as carbon sources during the optimization of the experimental conditions. *Allium cepa* seeds germination assay corroborated to certify the efficiency of the treatment process proposed, thus proving the attenuation of soil toxicity.

## 2. Materials and methods

### 2.1. Soil sampling

Sampling was carried out at Mina Chico Rei, located in Ouro Preto, Brazil ( $20^{\circ} 38'62.25''$  S,  $43^{\circ} 49'8255''$  W). Samples were dried at room temperature and homogenized. The particle size distribution was determined accordingly to the NBR7181 (1984). Water-soil suspensions 1:2.5 (w/v, distilled water) were agitated for 30 min stirring for pH and Eh measurements. Samples were digested with a 2:1  $\text{HNO}_3$ : HCl mixture before chemical analyses by inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 725). Arsenic quantification and As toxicity assessment experiments utilized only the solid particles with a diameter between 0.105 and 0.250 mm.

### 2.2. Microbial consortium

#### 2.2.1. Culture enrichment

The microbial consortium was obtained from sediments collected from an urban pond (Lagoa do Gambá, Ouro Preto, Brazil) enriched with Postgate B medium under low oxygen pressure. The microbial culture was adapted to aerobiosis after several successive subculturing transfers. During the adaptation steps, Postgate C and McKeen media were also used to assess cell growth and biosurfactant production. The cultures obtained using the Postgate B medium were selected for the subsequent studies.

#### 2.2.2. Identification

Cells were harvested by centrifuging ( $15720 \times g$ , 30 min,  $20^{\circ}\text{C}$  -ThermoFisher Heraeus Multifuge X1R) and washing with sodium chloride 0.9% (w/v). The pellet was frozen at  $-20^{\circ}\text{C}$  until analysis. DNA was extracted by using DNeasy PowerSoil (QIAGEN<sup>®</sup>) kit, following the manual instructions, which basically apply a physical-chemical cell disruption followed by column purification giving a DNA extract free of potential contaminants. DNA quality (A260/A280 ratio) and quantification was assessed in a NanoDrop (Thermo Fisher Scientific, USA) spectrophotometer. The 16S ribosomal DNA gene (16S rDNA) was partially amplified in Polymerase Chain Reaction (PCR) using the universal oligonucleotides (Fw GTGCCAGCMGCCGCGGTAA and Fr CCGTCAATYTTTTRAGTTT (Fouhy et al., 2016) to amplify the V4 – V5 region giving 385pb in size. The 30  $\mu\text{L}$  volume used for PCR reaction contained 3  $\mu\text{L}$  de Buffer (10 x); 1, 13  $\mu\text{L}$  dNTP (10 mM); 1, 5  $\mu\text{L}$  de  $\text{MgCl}_2$  (50 mM); 4, 5  $\mu\text{L}$  of each oligonucleotide (1  $\mu\text{M}$ ), 20 ng de DNA template and 0.48  $\mu\text{L}$  of Platinum<sup>®</sup> Taq (Invitro gen) (5U/ $\mu\text{L}$ ). The reaction was performed in a Thermocycler (Bioicycler<sup>®</sup>) in the following thermal cycles program: 5 min at  $94^{\circ}\text{C}$  followed by 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 1 min at  $57^{\circ}\text{C}$ , and 1 min at  $72^{\circ}\text{C}$ , and a final extension for 5 min at  $72^{\circ}\text{C}$ . According to the manufacturer's instructions, the amplicons were purified using the PCR Purification Kit (QIAquick<sup>®</sup>) and evaluated for integrity by agarose gel electrophoresis (0.8% agarose gel in TAE buffer, containing ethidium bromide) observed at UV light. The quantification of purified DNA amplicon used a Qubit dsDNA HS<sup>™</sup> (High Sensitivity) kit more specific than UV absorbance. Standards and samples were prepared according to the manufacture's description using the buffer provided and read the concentration using the Qubit 2.0 fluorometer (Life Technologies, USA). After that, the samples were normalized to 70 pM. 25  $\mu\text{L}$  of DNA amplicon was loaded in a 318 v2 chip (Life Technologies, USA) and run in NGS platform Ion PGM (Life Technologies, USA) using the facilities of the Genomic Laboratory on Biological Sciences Research Nucleus (NUPEB) at the Federal University of Ouro Preto. Sequences were analyzed in the Ion Reporter<sup>™</sup> software (Thermo Fisher Scientific).

### 2.3. Biosurfactant production

The consortium cells (1g) were inoculated in 500 mL salt medium containing ( $\text{g.L}^{-1}$ ):  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.50), KCl (0.10),  $\text{CaCl}_2$  (0.01),  $\text{NaNO}_3$

(7.00), FeSO<sub>4</sub>·7H<sub>2</sub>O (0.01), K<sub>2</sub>HPO<sub>4</sub> (1.00), yeast extract (0.01); glucose, 10% (w/v) and, glycerol, 1.0% (w/v); pH 7.0. The consortium was incubated aerobically at 30 °C for seven days on a rotary shaker at 200 rpm (Quimis, 0816M20). The cell-free supernatants containing biosurfactants were obtained from the centrifuged (ThermoFisher Heraeus Multifuge X1R, 15720 x g, 20 °C) broth supernatant.

#### 2.4. Effect of growth conditions and nutritional parameters

The effect of the glucose and glycerol (Glu/Gly) ratios on BS production, as well as the other initial growth parameters, were estimated. Biosurfactant production at different growth conditions: pH (5.5–9.5), temperature (25, 30, 35 °C), Glu/Gly ratios (0.5, 1.0, 1.5, 2.0, 2.5) and, the time of incubation (1–7 days) was assessed considering a univariate analysis, i.e., one variable changes while the others were kept constant (pH 7.5, 30 °C, Glu/Gly 1.0, 3 days). Details are described in the supplementary material (Data In Brief). The BS production was estimated indirectly by measuring the surface tension and the emulsification index (EI<sub>24</sub>) of the cell-free supernatants.

#### 2.5. Biosurfactant extracts characterization

The surface tension of the cell-free extracts was estimated using a Du Nouy tensiometer (25 °C), and the results express the average of seven measurements. The emulsification activity was assessed by the methodology described by Cooper and Goldenber (1987). According to this protocol, 2 mL of paraffin and 2 mL of cell-free medium (supernatant) were transferred to a glass test tube and agitated vortexing vigorously for 1 min. The vial was left to stand undisturbed for 24h. The emulsion stability and the emulsification index were calculated by dividing the measured height of the emulsion layer by the total height of the mixture, multiplying by 100. Surface tension and emulsification index values were obtained at pH 3, 7, and 11 (adjusted with NaOH, 1 mol.L<sup>-1</sup> and/or HCl, 1 mol.L<sup>-1</sup>).

The stability of the cell-free supernatants at different values (pH 3, 7, and 11) after exposure to low temperature was also evaluated. For this, 40 mL of the cell-free supernatants were frozen under -18 °C for 24, 48, and 72h in 50 mL Falcon tubes. Samples were defrosted at room temperature before use. The surface tension of the cell-free supernatants was measured as described previously.

#### 2.6. Column extraction

Several liquid extractants were tested for arsenic solubilization. Distilled water, SDS 1% (w/v), saponin 0.1% (w/v), CaCl<sub>2</sub> 0.1 mol.L<sup>-1</sup>, the biosurfactant extracts from cultures of different Glu/Gly (0.5, 1.0, 1.5, 2.5) and the extract of the determined optimum production condition (OPC). A set of (7.3 × 1.6 cm) polypropylene columns packed with 5.0g of solids was percolated with the chosen extractant solution (10 mL). After an initial cycle of 30 days, columns were eluted in 3 cycles of 7 days. The effect of pH (3, 7, and 11) on the arsenic extraction was evaluated only for the microbial produced biosurfactants extracts. The most efficient extractant solution was selected for a soil-flushing assay using a bigger dimension apparatus. With that purpose, an 18.5 cm long polypropylene column with a 3.8 cm internal diameter was packed with 75.0 g of the contaminated soil and used for a sequential extraction procedure consisting of seven 24 h leaching cycles. During the first cycle, a 100 mL aliquot of the extractant was transferred to the column for soaking the solid material. The liquid was eluted after 24h. Extractant aliquots of 75 mL were used six times for the sequential cycles. The column effluents were collected, and the pH and Eh were measured. The chemical composition of the eluted solutions was determined by ICP-OES (Varian®, 725/ES) after centrifugation (ThermoFisher Heraeus Multifuge X1R, 15720 x g, 20 min), filtration (0.45 µm cellulosic membrane) and, acidification (1% HNO<sub>3</sub>).

#### 2.7. Ecotoxicity assessment

Ecotoxicity tests followed the *Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test* from the *Guidelines for the Testing of Chemicals procedures*, from the Organization for Economic and Co-operation and Development (OECD, 2006) utilizing the *Allium cepa*, Baia Periforme variety (Feltrin®, 100% purity, Safra, 2012/2013). Each experimental set consisted of polyethylene flasks filled with 5,0 g of solid mixtures and ten seeds per vial. Solid combinations (20, 50, 80% w/w) were made with untreated or treated soil samples, collected after the column extraction procedure. Soil mixtures and the non-polluted soil were watered with distilled water or the liquid eluted from the soil washing columns, respectively. The experiment included a positive growth control containing a non-polluted solid substrate soaked with water. The seeds/-seedlings were allowed to grow for 21 days at room temperature under controlled humidity and temperature. After the growing time, the germination index was calculated, and the aerial structures and roots of the seedling were measured. Experiments were carried out in quadruplicates.

### 3. Results and discussion

#### 3.1. Properties of the mine tailing sample

Mine tailings were classified as sandy soil due to their particle size distribution and porosity (ABNT, 1995). The organic matter content of the mine tailings is less than 5 mg.kg<sup>-1</sup>. Therefore, the content of nutrients associated with the organic matter is scarce and insufficient for the development of many plant species (Wong, 2003). The pH measured was 3.49, which is not surprising when it comes to gold mining tailings. Furthermore, samples presented a high concentration of As, 4598 mg.kg<sup>-1</sup>. Not only arsenic but iron was found at a very high concentration (87779 mg.kg<sup>-1</sup>). Once more, that finding is not unusual if we consider the characteristics of the sampling points. In that area, the presence of banded-iron formations with hematite and silicates, along with quartzite (Alkmim and Teixeira, 2017), are well-known. Equally high contents of Mn (5766 mg.kg<sup>-1</sup>) and Al (3683 mg.kg<sup>-1</sup>) were confirmed. The high metal concentration of the soil samples collected indicates the high polluting potential of this material; hence some of the potentially toxic elements (PTE) found in the samples could be released into the environment, causing the contamination of several environmental compartments, mainly water. The soil chemical characteristics also unveiled the need for remediation and controlled mobilization of the most toxic metals, especially the As, using less toxic chemicals and an environmentally friendly approach (see Table 1).

#### 3.2. Microbial identification

The 16S rDNA sequencing analysis has revealed the microbial consortium was composed mainly of bacteria belonging to the Proteobacteria phylum, specifically those from two taxonomic orders: Xanthomonadales (98%) and Pseudomonadales (2%). The most abundant microbial genera found in the consortium was *Stenotrophomonas* sp, accounting for 74.5% of the identified microorganisms. Two species of this genus were identified: *Stenotrophomonas malthophilia* and *Stenotrophomonas geniculata*. Bacteria belonging to the *Stenotrophomonas* genus are of interest due to their bioproducts (biocides and biosurfactants) and heavy metals tolerance (Patil et al., 2012; Deepali et al., 2014; Singh et al., 2015; Gargouri et al., 2017; Matos et al., 2018; Larik et al., 2019; Tripathi et al., 2019). The second most abundant genera, *Pseudomonas* sp, accounting for 18.6% of the total identified sequences. *Pseudomonas* species are recognized by their ability to produce biosurfactants potentially useful for environmental applications (Chellaiah, 2018; Juwarkar et al., 2008; Sarin and Sarin, 2010; Tripathi et al., 2019). The identified species were *Pseudomonas aeruginosa* and *Pseudomonas hibiscicola*. This second organism has been intensely studied by the literature (Juwarkar

et al., 2007; Sun et al., 2019). The remaining genera identified in the consortium correspond to 0.14% of the total sequences.

Therefore, the microbial sequencing results pointed to the presence of biosurfactant producers. Hence, it is reasonable to presume the existence of a mixture of biosurfactants in the cell-free extracts. This mixture may consist probably of glycolipids and rhamnolipids. Thus, the results demonstrated the advantage of using mixed instead of pure cultures.

### 3.3. Biosurfactant production

Medium composition and other growth parameters have a strong influence on microbial metabolism as well as on the chemical composition of the products resultant from their growth, likewise for pure or mixed or cultures. In a consortium, the synergistic and metabolic interactions between the different microbial species also influence those processes. This paper aimed to estimate the effect of the different ratios of glucose and glycerol in the culture medium on both the emulsification index ( $EI_{24}$ ) and the surface tension. For this, the initial temperature (30 °C), incubation time (3 d), and pH (7.5) were kept constant. The BS production was confirmed by the use of more than one technique, as indicated by Satpute et al. (2008). The cell-free supernatants reduced water's surface tension ( $69 \pm 2 \text{ mN.m}^{-1}$ ) to approximately  $30 \text{ mN.m}^{-1}$ . Respectively, the supernatants R0.5, R1.0, R1.5, R2.0 R2.5 presented surface tensions  $32 \pm 0.83$ ,  $30 \pm 1.22$ ,  $35 \pm 0.70$ ,  $31 \pm 0.50$  and  $30 \pm 0.71 \text{ mN.m}^{-1}$ . The emulsification indexes ( $EI_{24}$ ) between 31 and 45% detected proved the emulsifying properties of the biological extracts.

The cell-free supernatants obtained from single carbon source cultures presented lower EI values compared to the ones with elevated concentrations of glycerol or glucose (Glu/Gly 0.5 and 2.5). The increase of biosurfactant yield when utilizing more than one source of carbon to the medium was verified for *Torulopsis bombicola* (Cooper and Paddock, 1984) and *Rhodococcus* spp cultures (Mutalik et al., 2008). Contrarily, no conclusions were reported about the effect of using two or more carbon sources on *Pseudomonas* and *Stenotrophomonas* species development and BS production (Mutalik et al., 2008). Replacing carbon sources affect the microbial metabolic routes and final bioproducts. The chemical composition of biomolecules, including the microbially produced biosurfactants, could be diverse. Thus, the changes aiming for metabolic improvements could also lead to the production of biosurfactants with improved surface-active properties.

For the 2.5 Glu/Gly cell-free supernatant, the initial pH 9.5 medium was shown to be slightly superior  $EI_{24}$ , 60%, after three days of incubation at 25 °C. Considering the environmental applications, shorter times of incubation with an increase in the surface-activity are more attractive. Thus, due to the difference of four days between the systems and the properties of surface-activity observed, the optimum production condition (OPC) was established: pH 9.5, Glu/Gly ratio of 2.5, 3 days of incubation at 25 °C.

**Table 1.** Main physicochemical properties of the mine tailing.

Parameter	Mine tailing	Fraction 0,105< $\phi$ < 0,250 mm
pH	3.48	3.49
Eh	+479.9 mV	+208.8mV
Porosity	43%	43%
CEC (cmol.kg <sup>-1</sup> )	9.88	8.04
Organic matter content	<5 mg.kg <sup>-1</sup>	<5 mg.kg <sup>-1</sup>
As (mg.kg <sup>-1</sup> )	4598	2760
Fe (mg.kg <sup>-1</sup> )	87779	52903
Mn (mg.kg <sup>-1</sup> )	5766	3485
Al (mg.kg <sup>-1</sup> )	3683	2365

### 3.4. Biosurfactant stability

Figure 1 depicts the stability of the cell-free supernatants at different pH and the discrete variations observed on surface tension and  $EI_{24}$ . The maintenance of the surface activity of the cell-free supernatants at different pH values is an advantage since it allows the use of the BS extracts under diverse conditions either during laboratory-scale tests as well as during real applications. Some authors discussed it before and mentioned some biosurfactants are unstable when exposed to extreme pH values, especially the acidic pH (Kim et al., 2000; Wang and Mulligan, 2009b).

The emulsions produced with the supernatants obtained from cultures kept at pH 3.0 were less stable markedly for the extracts obtained with the higher Glu/Gly ratio (Figure 1-D). In that case, the emulsifying capacity of the extract was reduced to less than 50% of the observed under alkaline growth conditions. Moreover, for those extracts produced at the optimized conditions (OPC), the lowest  $EI_{24}$  was observed for the biological extracts from cultures kept at neutral pH (Figure 1-E), with a slight reduction of 10%. The surface tension results for all supernatants, despite the pH, were around  $30\text{--}40 \text{ mN.m}^{-1}$ , thus indicating pH does not affect this characteristic of the biological extracts.

The cell-free supernatants were frozen and defrosted to estimate the effect of these temperature changes on the surface activity. The results proved the biosurfactant containing extracts were sensitive to the low temperatures (Supplementary in Material – Data in Brief - Figure 1). Supernatants produced using 0.5, and 1.5 Glu/Gly ratio cultures were more affected by the decrease in temperature, presenting an average increase of  $20 \text{ mN.m}^{-1}$  on the surface tension. In contrast, the supernatant produced with the Glu/Gly ratio of 1.0 was more stable, regardless of the freezing time. In this way, an increase in surface tension and a decrease of  $EI_{24}$  after the procedure could indicate the precipitation of the biosurfactants or other structural effects. Despite acidification (pH < 4), followed by cooling, is a well-known strategy to purify biosurfactants (Weber and Zeiner, 2014), this approach is not the best choice, however, for the purification of the BS produced, as described here. In this case, freezing either for storage or purification purposes might affect the surface activity of the bioproducts.

### 3.5. Sequential extraction of arsenic

#### 3.5.1. Extractants evaluation

The efficiency of each different extractant for As removal was evaluated individually. Distilled water removed 0.04%, of the initial As content while  $\text{CaCl}_2$   $0.1 \text{ mol.L}^{-1}$ , SDS  $1\% (\text{m.v}^{-1})$  and Saponin  $0.1\% (\text{m.v}^{-1})$  solubilized, respectively, 0.03%, 0.8% and 0.05% of the As after 51 days of sequential extractions (4 cycles). Although the extraction efficiencies of all the extractants are negligible, it is necessary to reinforce that the As content of the solid material is  $2760 \text{ mg.kg}^{-1}$ . Therefore, even an ordinary event like rainwater flow on the soil surface would be enough to leach a considerable amount of arsenic. In that case, the leaching of no more than 0.04% of the As could produce As-enriched drainages with a soluble As content at least ten times the maximum limits determined for potable water (WHO, 2017). Therefore, the alert about the polluting potential of that tailing arises.

The cell-free supernatants showed the highest As removal efficiency amongst the extractants evaluated. The cumulative As removal efficiency increased with the Glu/Gly content as demonstrated for the cell-free extracts R0.5, R1.0, R1.5, and R2.5, which removed 7.67, 8.13, 8.71, 9.24%, respectively. The highest cumulative removal for the toxic element was obtained for the OPC supernatant (9.78%). Therefore, the correlation between biosurfactant production and arsenic solubilization was confirmed.

#### 3.5.2. Effect of pH in as mobilization

The investigation of how pH influences As extraction is an essential issue since the pH may affect the equilibrium of the whole system. This

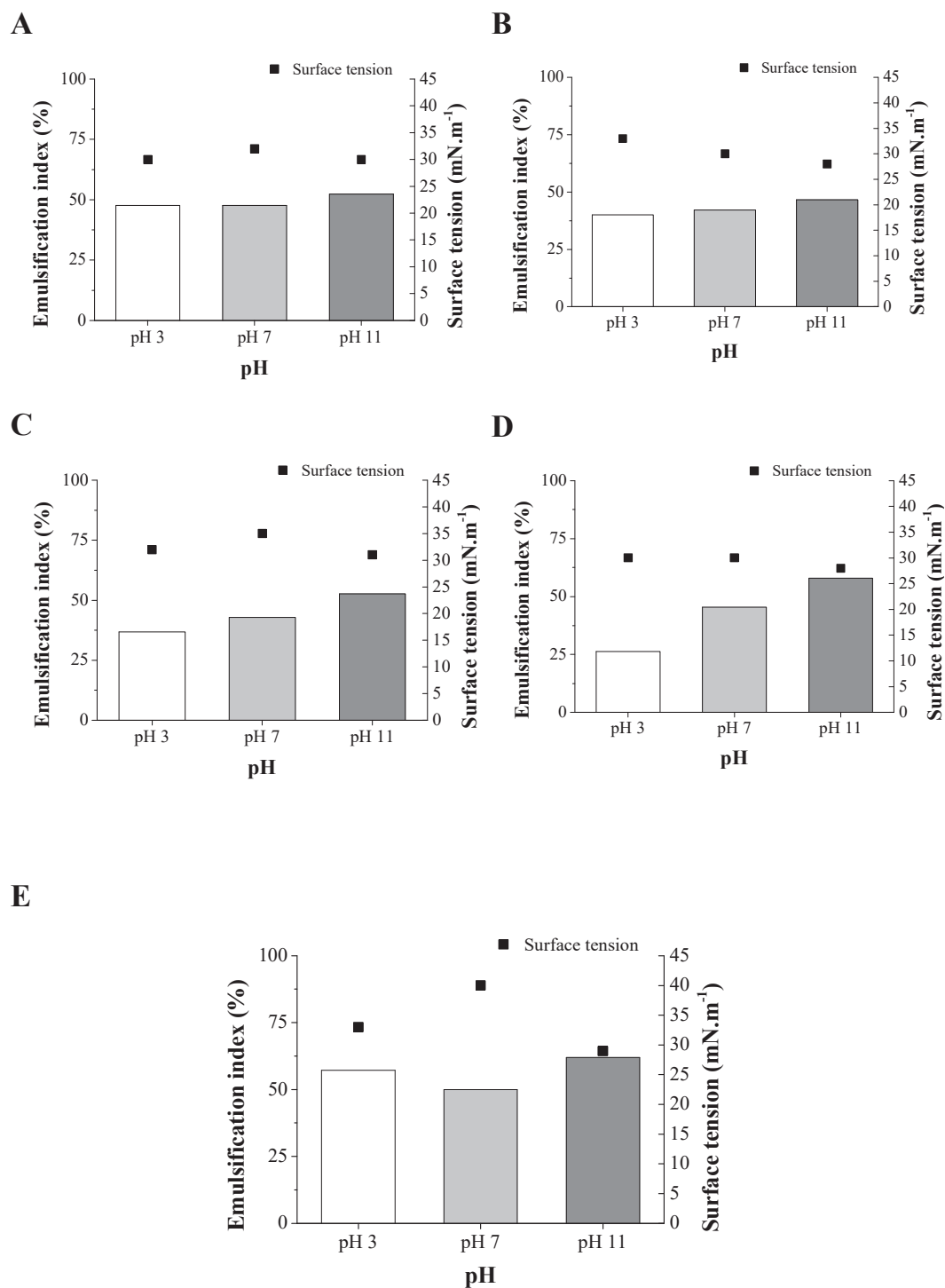
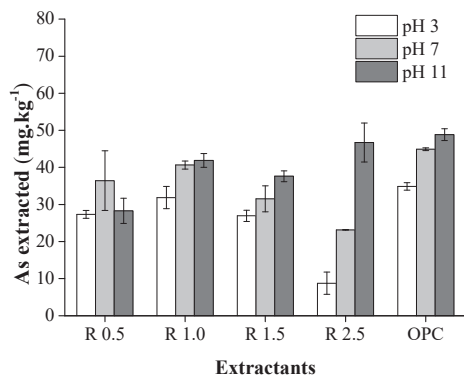


Figure 1. Effect of pH on the surface activity properties of the cell-free supernatants Glu/Gly ratio A – 0.5. B – 1.0. C – 1.5. D – 2.5. E – OPC.

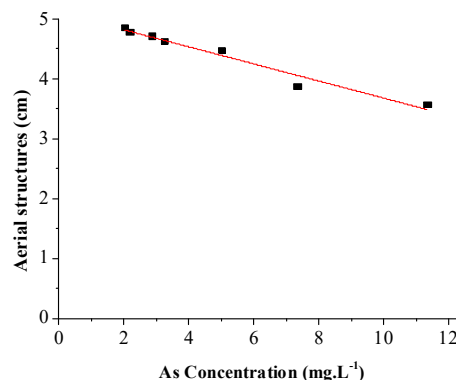
parameter could affect not only the stability of the potentially toxic elements (PTE) but also the chemical properties of the biosurfactants. The medium pH directly influences the speciation and solubility of PTE. The surface properties of the extractants and the extractant-metal interactions could be equally impacted by changing the pH. Thus, aiming to assess the effect of this parameter on arsenic solubilization, the cell-free extract pH was adjusted to 3, 7, and 11 for the extraction procedures conducted at room temperature. Figure 2 depicts the results obtained following this experimental approach. The extraction increased as a function of the pH. The most expressive arsenic extractions were obtained at pH 11, except for the cell-free extract obtained with the lower Glu/Gly ratio (R0.5). For

this cell-free supernatant, pH 7 led to the highest As removal ( $36 \text{ mg.kg}^{-1}$ ). The sensitivity to acidic conditions affects both the properties and the As removal in the R2.5. The OPC-extract achieved the most expressive removal at pH 11 ( $49 \text{ mg.kg}^{-1}$ ). A similar effect on arsenic removal efficiency at pH 11 was reported previously by others (Wang and Mulligan, 2009a; Arab and Mulligan, 2018). In that case, the experimental approach was a rhamnolipids-based soil flushing using *Pseudomonas aeruginosa* culture bioproducts. The OPC extractant at pH 11 was chosen for the subsequent experiments due to the experimental results.

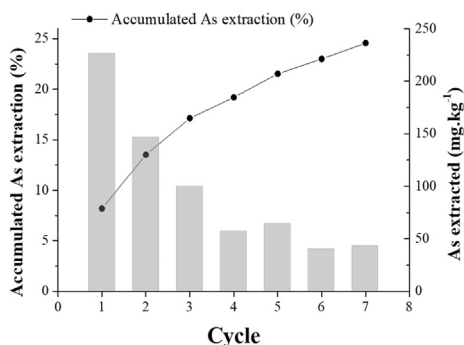
Arsenic and other metals/metalloids mobilization are a consequence of many complex chemical interactions, and three main mechanisms



**Figure 2.** As extraction by different extractants of 0.5, 1.0, 1.5, 2.5 Glu/Gly ratio, and OPC in pH 3, 7, and 11. The error bars represent the standard deviation of replicates.



**Figure 4.** Correlation between As concentration ( $\text{mg.L}^{-1}$ ) and the aerial structures (leaves and stem) of *Allium cepa* seedlings.  $Y = -0.1427 + 5.1035; R^2 = 0.9589$ .



**Figure 3.** Arsenic mobilization from the mine tailing by the biosurfactant extract (BS) at pH 11. Bed volume = 75mL.

could explain those phenomena. The first hypothesis assumes the arsenic desorption from the soil as a result of interfacial tension reduction in the liquid-solid surface of particles so, the interaction between the biosurfactant monomers and As occurs, followed by the incorporation and stabilization of the element in the center of the formed micelles (Wang and Mulligan, 2009b; Sarubbo et al., 2015). Moreover, the arsenic ion can also be mobilized indirectly by the interaction with another metal, which, by its turn, interacts directly with the BS molecule through a mechanism denominated metal-bridging. Another proposed mechanism suggests the desorption of labile As species of the soil and its

complexation. This mechanism relies on the Le Chatelier equilibrium principle (Miller, 1995), and the last premise assumes the BS is anionic and, therefore, arsenic ions could be mobilized by an ionic exchange mechanism (Santos et al., 2016).

**3.5.3. Optimized extraction leaching column**

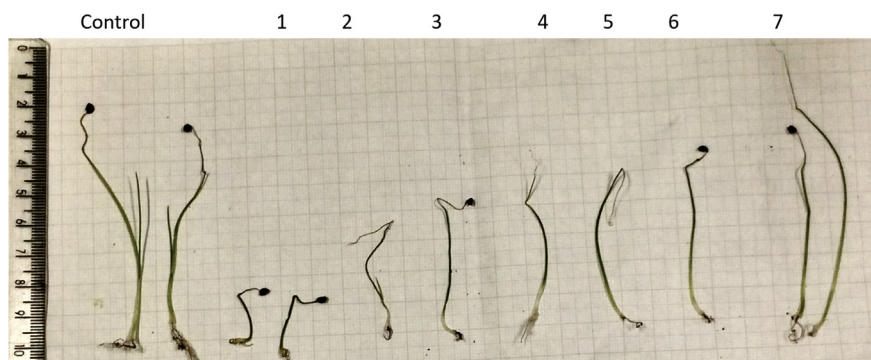
The soil flushing assessment aims to simulate substance extraction by leaching mechanisms to predict the trends of treatment systems. Figure 3 depicts the results obtained using the 18 cm column. After 7/24h cycles, approximately 24.6% of the initial As content was leached, corresponding to  $678.39 \text{ mg.kg}^{-1}$ . About 3.7 and 6.6% of the Al and Mn from the tailing were also leached, respectively. It is indicative of the possible involvement of these elements in As mobilization since the natural occurrence of arsenic in the mineral structure of manganese and aluminum oxides is likely to occur, and the dissolution of these oxides is relevant for As mobilization in the environment (Smedley and Kinniburgh, 2002). Not only arsenic but other metallic compounds, mainly Zn, Cu, Fe and, Pb oxides, are reported to be dissolved by biosurfactants. That evidence reinforces the most probable dissolution mechanism is the anion exchanging of As and other elements by the anionic biosurfactant (Gusiatin, 2014; Wang and Mulligan, 2009b).

**3.6. Ecotoxicity assessment**

The bioassay performed could either evaluate the soil's polluting potential and the evolution of the remediation process. The seedlings

**Table 2.** Dimensions of aerial structure and root of seedling and germination index of seeds of *Allium cepa* in the ecotoxicity assay. The variation corresponds to the standard deviation of the replicates.

Sample	Aerial structure (cm)		Root (cm)		Germination Index (%)
	Media	Median	Media	Median	
Control	7.47 ± 0.51	8.00	1.42 ± 0.66	1.32	70
Untreated soil 20%	2.18 ± 3.08	2.13	0.89 ± 1.26	0.00	7.5
Untreated soil 50%	0.80 ± 0.00	0.80	0.23 ± 0.33	0.00	2.5
Untreated soil 80%	0.00	0.00	0.00	0.00	0
1 Cycle	3.57 ± 1.08	3.76	0.90 ± 0.31	0.63	40
2 Cycle	3.87 ± 0.90	4.06	0.77 ± 0.06	0.70	42.5
3 Cycle	4.47 ± 0.34	4.09	1.16 ± 0.25	0.99	45
4 Cycle	4.71 ± 0.95	4.62	0.57 ± 0.11	0.55	52.5
5 Cycle	4.63 ± 0.60	4.23	0.93 ± 0.26	0.83	50
6 Cycle	4.86 ± 1.46	4.94	0.41 ± 0.11	0.36	55
7 Cycle	4.78 ± 1.72	4.91	0.67 ± 0.18	0.70	55
Treated Soil 20%	6.97 ± 0.41	7.23	1.46 ± 0.56	1.49	60
Treated soil 50%	7.02 ± 1.44	7.05	1.24 ± 0.30	1.13	57.5
Treated soil 80%	4.29 ± 0.79	4.18	1.19 ± 0.24	1.04	52.5



**Figure 5.** *Allium cepa* seedling development after watering non-contaminated soil with the soil-flushing eluted solutions. Numbers are indicative of the cycles carried out (bed volumes). Control set soaked with distilled water.

watered with the column's effluent presented visible morphological abnormalities characterizing the retarded development, confirmed in comparison with the control (Figure 5). The results for the control systems containing 20, 50, and 80% of tailing demonstrate the influence of the soil in the germination and seedling growth (Table 2). The germination index reduction with the increase of the untreated soil concentration in the systems, respectively, 7.5, 2.5, and 0%, confirmed the toxic effect of soil components. The main effects observed were low stages of seedling development with reduced aerial structures and roots.

Arsenic is a non-essential element for plants, and its toxicological potential through different mechanisms is described in the literature (Ghosh and Sil, 2015; Singh et al., 2011). Due to the similarities with the  $\text{PO}_4^{3-}$ , the ion arsenate ( $\text{AsO}_4^{3-}$ ) can replace phosphate in diverse cell reactions, causing toxic effects in seedling and retardation of the development of plant structures (Mahmood, 2010; Strawn, 2018). The main factors related to the toxicity of metal and metalloids are the concentration of the potentially toxic elements (PTE), the ionic activity, and the kinetics of the reactions which may occur in the soil. Consequently, the impact on the growth of vegetables is related to the soluble, exchangeable, organic, and insoluble fractions of As. Thus, the residual, mineralized, and silicate incorporated portions in the tailings analyzed are strongly related to the geological background of the region. Table 2 summarizes the results obtained from the ecotoxicological assay, which aimed to evaluate the effect of the soil properties and the high concentrations of As over the *Allium cepa* plants. The *Allium cepa* assay is useful for detecting the deleterious effects of a wide variety of environmental pollutants such as metals, pesticides, aromatic hydrocarbons, and others (Leme and Marin-Morales, 2009).

The seeds system watered with the 75 mL column effluent had the toxicity and the pollutant potential of the soil assessed by this method. Meanwhile, the negative control presented a 70% germination index, and the GI varied from 40 to 55% in the systems watered with the effluents of the first and seventh cycles, respectively. Regardless of the plants, roots are the structures the first exposed to the toxic elements in the soil. Therefore, a consequence of As exposure is the inhibition of root proliferation and extension. Consequently, metabolic processes are altered, and the growth of the whole plant structures are compromised (Abedin et al., 2002; Farnese et al., 2014; Finnegan and Chen, 2012). The morphological evaluation of the seedlings indicated different degrees of growth. Seeds development increased gradually from the beginning to the seventh cycle (Figure 4). From the morphological results, it is possible to correlate the As concentration in the solutions eluted from the soil-flushing cycles to the structures of seedlings (Figure 5). The results also demonstrate the efficient response of *Allium cepa* to short-time exposure to As contamination and can be used as an environmental tool of hazardous health prediction.

#### 4. Conclusion

This study assessed biosurfactant production by a microbial consortium and its efficiency in As mobilization. The optimized microbial growth condition, i.e., the growth condition which guarantees the higher biosurfactant production, was achieved. The microbial consortium obtained the best biosurfactant yield when growing in the liquid medium supplemented with a mixture of glucose and glycerol at a ratio of 2.5, at 25 °C for 3 days.

Experimental concepts, as well as the results discussed here, emphasize the role of biosurfactants in the remediation of toxic metals and metalloids. The biosurfactant efficiency in mobilizing arsenic from mining tailings in column soil-flushing tests was verified. The best arsenic solubilization results obtained through soil flushing were achieved with the use of the BS produced at pH 11. The application of the biosurfactant under alkaline conditions could enhance the As mobilization and improve the quality of soil properties. Hence, the use of this type of biological compounds aiming for the remediation of As contaminated soils, providing environmental benefits, and a practical approach of replacement of synthetic extractants is a reasonable assumption.

Additionally, using mixed microbial cultures containing different microbial species may lead to the production of diverse bioproducts, not only biosurfactants, and the advantages or the disadvantages of this approach needs a more meticulous investigation in the future. The nutritional needs of different microbial cultures deserve particular attention to guarantee the best conditions to optimize biosurfactant production.

Finally, the *Allium cepa* seeds in the ecotoxicological assay were useful as a hazard recognition tool for soil contamination due to the sensitivity and rapid response to diverse pollutant substances.

#### Declarations

##### Author contribution statement

M.C. Teixeira: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

L.S.S. Araújo: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

S.Q. Silva: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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#### Data availability statement

Data included in article/supplementary material/referenced in article.

#### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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