Contents lists available at ScienceDirect

Process Safety and Environmental Protection

journal homepage: www.elsevier.com/locate/psep

# Biosurfactant from *Candida sphaerica* UCP0995 exhibiting heavy metal remediation properties

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ARTICLE INFO

*Article history:*
Received 12 August 2015
Received in revised form 15 April 2016
Accepted 13 May 2016
Available online 19 May 2016

*Keywords:*
Biosurfactant
*Candida sphaerica*
Heavy metals
Soil washing
Wastewater
Remediation

ABSTRACT

The performance of an anionic biosurfactant from *Candida sphaerica* in the removal of heavy metals from soil collected from an automotive battery industry and from aqueous solution was evaluated. Multiple combinations of biosurfactant solutions, NaOH and HCl were tested. The results indicated removal rates of 95, 90 and 79% for Fe, Zn and Pb, respectively. The addition of HCl increased the metal removal rate when used with biosurfactant solutions at 0.1 and 0.25%. The use of the recycled biosurfactant after precipitation of the metals in the treated soil demonstrated the ability of the biomolecule to remove 70, 62 and 45% of Fe, Zn and Pb, respectively. Sequential extraction procedures were conducted to determine the speciation of the heavy metals before and after washing the soil with the biosurfactant. The biosurfactant was effective in removing the exchangeable, carbonate, oxide and organic fractions of heavy metals. Tests were performed to evaluate the conductivity and chelating activity of the biosurfactant in aqueous solutions containing Pb and Cd. Atomic absorption spectroscopy studies demonstrated metal removal at a concentration less than the critical micelle concentration. The biosurfactant washing technology is a promising alternative for the remediation of wastewater and soil contaminated with metals.



## 1. Introduction

Natural and anthropogenic activities alter the nature of the surface soil environment (Hsu et al., 2006; Ikenaka et al., 2010). During the last few decades, soil pollution has become one of the most serious public environmental issues, not only by organic pollutants, but also inorganic pollutants such as heavy metals (Liao et al., 2008; Adelekan and Abegunde, 2011; Wuana and Okieimen, 2011). An exceptionally high amount of heavy metal contamination is found in mining, smelting or landfilling of industrial waste soil, where the most frequently identified metals are Pb, Cu, Zn, Cd, Ni, Cr. A large number of metal-contaminated sites have been reported in many countries, such as the United States, European countries, Taiwan, India, and China (Maity et al., 2013). Research into effective elemental remediation processes for environmental clean-up has been the focus of growing attention (Gusiatin and Klimiuk, 2012).

Traditional remediation techniques used for the removal of heavy metal contamination include treatment of contaminated soils with water, inorganic and organic acids, chemical surfactants and metal chelating agents such as EDTA (Chakraborty and Das, 2014). However, these methods do not ensure proper removal of the contaminating metal ions from the soil. Remediation techniques proposed for contaminated soils like thermal treatment, stabilization,

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http://dx.doi.org/10.1016/j.psep.2016.05.010
0957-5820/

excavation and landfill, are of infrequent use because of land space requirements (Wang and Mulligan, 2009). For proper removal of the metals from a contaminated environment, a good metal complexing agent is required possessing the properties of solubility, environmental stability and good complexation potential for metals (Juwarkar et al., 2007). Microbial surface-active metabolites, called biosurfactants, are such metal complexing agents that have been reported to be effective in the remediation of heavy metal contaminated environments (Chakraborty and Das, 2014). There are many reasons that make biosurfactants promising alternative agents for remediation purposes. These are their less toxic nature (Sandrin and Maier, 2000), better environmental compatibility and biodegradability (Gao et al., 2012). Other advantages include their production from inexpensive agro-based raw materials and organic wastes (Masli and Maier, 2000; Chrzanowski et al., 2012) and retention of their activity even at extremes of temperature, pH and salt concentration.

In recent years, a number of biosurfactants have been proposed for the bioremediation of soil contaminated with heavy metals and studies have demonstrated the potential of these biological agents (Sarubbo et al., 2015). Rhamnolipids are the group of biosurfactants most cited in the literature for application in heavy metals removal. Lipopeptidic biosurfactants like surfactin have also been exploited as ion collectors in wastewater treatment (Sarubbo et al., 2015). In addition to these biosurfactants of bacterial origin, other types of biosurfactants (mostly sophorolipid in nature) produced by species of the genus *Candida* have also been successfully employed in the flotation of heavy metals (Albuquerque et al., 2012; Menezes et al., 2011; Rufino et al., 2011). Biosurfactants from plant origin have also been applied in the remediation of metals (Sarubbo et al., 2015).

Considering the satisfactory results obtained previously with the biosurfactant produced by *Candida sphaerica* in removing organic pollutants (Luna et al., 2013; Luna et al., 2015), the present study investigated the efficiency of this biosurfactant on remediation of heavy metal contaminated soil. In addition, sequential extraction procedures were used to determine which metal fractions could be more easily removed by the biosurfactant. Used biosurfactant obtained through a precipitation process was recycled for the subsequent soil treatment. The application of the biosurfactant in removing the heavy metals from heavy metal containing solutions was also investigated.

## 2. Materials and methods

### 2.1. Soil

The Physicochemical characterization of the soil used in this study followed standard methods (ABNT, 1984; EMBRAPA, 1997). The sample was obtained from an automotive battery industrial area contaminated for many years with metals and industrial waste discharged to the soil. Soil was air dried and sieved to remove coarse sand and stone using a 2 mm sieve. The soil samples were homogenized and stored in a plastic container for subsequent experiment. The heavy metal content of the soil was determined by acid digestion ($HNO_3$ + HCl) at boiling temperature (ASTM, 1993). The digested liquid was filtered with a 0.45 μm nitrocellulose membrane filter and filtrate was analyzed for heavy metals by atomic absorption spectroscopy (AAS).

### 2.2. Biosurfactant production

*Candida sphaerica* UCP 0995 was obtained from the culture collection of the *Universidade Católica de Pernambuco*, Brazil. The microorganism was maintained at 5 °C on yeast mould agar slants containing (w/v) yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), D-glucose (1.0%) and agar (5.0%). Transfers were made to fresh agar slants each month to maintain viability.

Two types of industrial waste were used as substrates to produce biosurfactants. Ground-nut oil refinery residue was obtained from ASA LTDA, Recife-PE, Brazil, and corn steep liquor from Corn Products do *Brasil*, Cabo de Santo Agostinho-PE, Brazil.

The inoculum of *C. sphaerica* was prepared by transferring cells grown on a slant to 50 mL of yeast mould broth. The seed culture was incubated for 24 h at 28 °C and agitated at 150 rpm. The yeast was cultivated in a submerged culture with shaking in a MA 832 shaker (Marconi LTDA., Brazil). The basal medium was composed of 9% groundnut oil refinery residue and 9% corn steep liquor dissolved in distilled water. The medium was sterilized by autoclaving at 121 °C for 20 min. The final pH of the medium was 5.3 and the surface tension before inoculation was 50 mN $m^{-1}$. The inoculum (1%, v/v) was added to the medium at the rate of $10^4$ cells $mL^{-1}$. Cultivation was carried out in Erlenmeyer flasks at 28 °C with shaking at 150 rpm for 144 h (Luna et al., 2013).

The culture broth free of cells was acidified with 6 M HCl to pH 2.0 and precipitated with two volumes of methanol. After 24 h at 4 °C, samples were centrifuged at 5000 g for 30 min, washed twice with cold methanol and dried at 37 °C for 24–48 h. The yield in isolated biosurfactant was expressed in g $L^{-1}$ (Luna et al., 2013).

### 2.3. Soil washing studies using biosurfactant

A series of washing was performed using the isolated biosurfactant at 0.1% concentration, 0.25% concentration and 2.5% concentration and using the cell-free broth (crude biosurfactant). Deionized water was used as the control. NaOH solution at 1% and HCl at 0.7%, as well as combinations of biosurfactant solutions and cell-free broth with 0.7% HCl or 1% NaOH as additives were also tested. 5.0 g of the contaminated soil were transferred to 125 mL Erlenmeyer flasks and 50 mL of the washing solution at the different concentrations described above were added. The samples were incubated in a rotary shaker (200 rpm) for 24 h at 27 °C and then were centrifuged at 5000 g for 10 min. The procedure was repeated three times. The supernatants were analyzed for metal concentration with an atomic absorption spectrophotometer (Perkin Elmer AAnalyst$^{TM}$ 800).

The kinetic study of heavy metals removal was conducted with the cell-free broth as described above. Samples were taken after 1, 3, 5, 7, 9, 11 and 13 days. The supernatants were analyzed for metal concentration by atomic absorption spectrometry.

### 2.4. Sequential extraction

The purpose of the sequential extraction studies was to determine the presence of metals in the sediments among the exchangeable, oxide, carbonate, organic and residual fractions. The harsher the chemicals required, the more difficult it is to remove the metals.