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ORIGINAL PAPER

Simultaneous phenanthrene and cadmium removal from contaminated soil by a ligand/biosurfactant solution

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Abstract Surfactants and inorganic ligands are pointed as efficient to simultaneous removal of heavy metals and hydrophobic organic pollutants from soil. However, the biosurfactants are potentially less toxic to soil organisms than other chemical agents. Thus, in this study the efficiency of combinations of iodide (I^-) ligand and surfactants produced by different bacterial species in the simultaneous removal of cadmium (Cd^{2+}) and phenanthrene in a Haplustox soil sample was investigated. Four microbial surfactants and the synthetic surfactant Triton X-100 were tested with different concentrations of ligand. Soil samples contaminated with Cd^{2+} and phenanthrene underwent consecutive washings with a surfactant/ligand solution. The removal of Cd^{2+} increased with increased ligand concentration, particularly in solutions containing biosurfactants produced by the bacterial strains *Bacillus subtilis* LBBMA155 (lipopeptide) and *Flavobacterium* sp. LBBMA168 (mixture of flavolipids) and Triton X-100. Maximum Cd^{2+} removal efficiency was 99.2% for biosurfactant produced by *Arthrobacter oxydans* LBBMA 201 (lipopeptide) and 99.2% for biosurfactant produced by *Bacillus* sp. LBBMA111A (mixed lipopeptide) in the presence of 0.336 mol iodide l^{-1}, while the maximum efficiency of Triton X-100 removal was 65.0%. The biosurfactant solutions removed from 80 to 88.0% of phenanthrene in soil, and the removal was not influenced by the presence of the ligand. Triton X-100 removed from 73 to 88% of the phenanthrene and, differently from the biosurfactants, iodide influenced the removal efficiency. The results indicate that the use of a single washing agent, called surfactant-ligand, affords simultaneous removal of organic contaminants and heavy metals.

Keywords Surfactant · Triton X-100 · Ligand · Heavy metal · PAHs

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Introduction

Heavy metals and hydrophobic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) are two of the most important and frequent soil and sediment contaminants. Significant quantities of heavy metals, including zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr) and cobalt (Co), along with PAHs and chlorinated hydrocarbons, are found in marine sediments (Jaffe et al. 2003) and in soils of industrialized urban areas (Ho and Hui 2001). Positive correlations between Cd and Pb and polychlorinated biphenyls (PCBs) and PAHs can be attributed to the existence of a common soil pollution source in regions where

chemical and steel industries are present (Weiss et al. 1994).

Heavy metals and hydrophobic organic compounds exhibit divergent chemical characteristics (Fernandes et al. 2009). Heavy metals are relatively mobile in the soil, especially at low soil pH, and are typically ionic in soil solution (Shin et al. 2004). The hydrophobic organic compounds such as PAHs are relatively insensitive to pH change and exhibit lipophilic properties (Shin et al. 2004). These differences in chemical characteristics make it difficult to use a single washing agent to simultaneously remove the two groups of contaminants.

Organic or inorganic acids and chelating agents are usually used in soil remediation procedures to remove heavy metals (Reddy and Chinthamreddy 2000; Wasay et al. 2000; Dacera and Babel 2008) while solvents or surfactants are more appropriate agents for the remediation of hydrophobic organic pollutants (Fava and Di Gioia 1998; Chu and Kwan 2003).

Currently, the strategy to simultaneously remove heavy metals and hydrophobic organic contaminants in soil includes use of leaching agents such as distilled water, humic acid, phospholipids and sodium dodecyl sulfate (SDS) (Schmelling et al. 1998; Hirner et al. 1998; Abramovitch et al. 2003). SDS is considered the most appropriate for solubilization of organic contaminants, given its ability to interact with hydrophobic compounds and hydrocarbon chains. However, the capacity of SDS to remove heavy metals (hydrophilic contaminants) has not been demonstrated (Hirner et al. 1998).

Recently, ligands were shown to change the character of wastewater heavy metals from hydrophilic to hydrophobic, thus enabling surfactants to simultaneously desorb both heavy metals and toxic organics from contaminated soils. Shin et al. (2000) showed that the nonionic surfactant Triton X-100, in combination with an inorganic ligand [iodide (I^-) or thiocyanate (SCN^-)], was capable of removing Cd, Cu and Zn from soil contaminated with heavy metals and PCBs. The authors proposed that the ligand forms a complex with the metal ion in the surfactant solution, reducing its hydrophilic character and allowing its absorption within the surfactant micelle. This behavior can lead to simultaneous desorption of heavy metals and organic hydrophobic compounds adsorbed to particles of soil and sediment.

Biosurfactants and ligands are potentially less toxic to soil organisms than other chemical agents such as synthetic surfactants, strong acids and chelating agents. However, no reports were found in the literature consulted on evaluation of efficiency of the combination of biosurfactants and ligands in the simultaneous mobilization of hydrophobic organic compounds and heavy metals. Thus, this work aimed to evaluate the efficiency of simultaneous removal of phenanthrene and cadmium from an oxisol (Haplustox) with combinations of iodide ligand and biosurfactants produced by different bacterial species.

Materials and methods

Surfactants

Five types of surfactants, the synthetic nonionic surfactant Triton X-100 ($C_8H_{17}C_6H_4$ $(OC_2H_4)_{10}OH$) and four biological surfactants produced by bacterial strains *Bacillus* sp. LBBMA 111A (mixed lipopeptide), *Bacillus subtilis* LBBMA 155 (lipopeptide), *Flavobacterium* sp. LBBMA 168 (mixed of flavolipids) and *Arthrobacter oxydans* LBBMA 201 (lipopeptide) were tested. The biosurfactants were produced in the laboratory of Biotechnology and Biodiversity for the Environment (LBBMA) of the Department of Microbiology, Federal University of Viçosa, and isolation was performed using the techniques of acid precipitation, ultrafiltration and thin-layer chromatography (TLC). The surface active properties of each surfactant are described in Table 1.

Surfactin, iturin and fengycin of *Bacillus* sp. LBBMA 111A, surfactin of *B. subtilis* LBBMA 155 and arthrofactin of *A. oxydans* LBBMA 201 were produced in mineral medium supplemented with 2% glucose (w v^{-1}) and 2% hexadecane (v v^{-1}) (added after glucose exhaustion). Flavolipids of *Flavobacterium* sp. LBBMA 168 were produced in drop-collapse mineral medium (Bodour and Miller-maier 1998) containing 2% glucose (w v^{-1}) as sole carbon source. Cultures were grown in flasks at 30°C in an orbital shaker (New Brunswick Scientific) under constant agitation (200 rpm) for 7 days.

Isolation and purification of biosurfactants were performed after removal of cells by centrifugation (12,000×*g*, 20 min, 25°C), followed by filtration using a 0.45 μm pore size membrane filter.

Table 1 Critical micelle concentration (CMC) e surface tension at CMC (γ_{CMC}) of surfactants used in this study

Surfactant type	Abbreviations	γ^{a}_{CMC} (mN m^{-1})	CMC (mg l^{-1})
Lipopeptides	LBBMA 111A	33.9	150
Lipopeptide	LBBMA 155	34.6	180
Flavolipids	LBBMA 168	38.8	200
Lipopeptide	LBBMA 201	36.6	200
Triton X-100	–	39.0	268
Water	–	72.0	–

LBBMA 111A Surfactant produced by *Bacillus* sp. LBBMA 111A, *LBBMA 155* Surfactant produced by *Bacillus subtilis* LBBMA 155, *LBBMA 168* Surfactant produced by *Flavobacterium* sp. LBBMA 168, *LBBMA 201* Surfactant produced by *Arthrobacter oxydans* LBBMA 201, *Triton X-100* Synthetic surfactant

[a] Surface tension regarding the critical micelle concentration (CMC)

Biosurfactants LBBMA 111A, LBBMA 155 and LBBMA 201 were acid precipitated, after adjusting the pH of the medium to 2.0 with 12 M HCl. Biosurfactants pellets were recovered by centrifugation (12,000×*g*, 20 min, 20°C) (Vater et al. 2002) and dissolved in water at pH 7.0. The products were extracted with chloroform and methanol, filtered with qualitative filter paper, and, the solvent evaporated, submitted to TLC on silica gel 60 F254 (VETEC) plates and quantified. Biosurfactant LBBMA 168 was recovered using an ultrafiltration cell (Amicon model) equipped with 500 and 1000 Da molecular weight cutoff membranes (Lin et al. 1998), followed by TLC and quantification. The compounds plotted on the TLC plates were visualized under ultraviolet light (UV 254 nm) and revealed by ninhydrin (0.2%, w v^{-1}) (Riedel–de–Haen, Seelze, Germany) and 3 M H_2SO_4 (v v^{-1}), followed by heating the plates at 100°C for 5 min (Horowitz et al. 1990). The impure fractions were separated by preparative TLC on glass plates (20 × 20 mm) pre-coated with silica gel 60 F_{254} (VETEC) and eluted in mixtures of chloroform and methanol. The bands on the TLC plates were detected under UV light (254 nm), and those corresponding to compounds of interest were scraped from the plates, pooled, and extracted three times with chloroform.

Surfactant concentrations in the washing solutions were equivalent to two times their critical micelle concentration (2CMC), except for Triton X-100, which was used at a concentration (0.05 mol l^{-1}) well above its CMC (Table 1). Potassium iodide (KI) was the source of iodide ligand and phenanthrene was used as PAH model compound. The washing agents, including the surfactant and ligand, were prepared in distilled water, adjusted to pH 6.8 with NaOH.

Soil

Soil used in this study was collected from a 0–20 cm deep layer in Viçosa (Minas Gerais, Brazil) and was classified as Haplustox (Soil Survey Staff 1999). Its physical and chemical characterization was carried out in the Soil Department Routine Analysis Laboratory of the Federal University of Viçosa. The organic carbon content was quantified by wet oxidation of organic matter, using potassium dichromate solution in acid medium (Yeomans and Bremner 1988). The cation exchange capacity (CEC) was determined by measurement of Ca^{2+}, Mg^{2+} and Al^{3+} desorbed in the soil, after saturation with a solution of 0.1 mol l^{-1} $BaCl_2$ (Hendershot et al.1993). This soil presented high clay content, average organic matter content and high acidity (Table 2). Clayey soils tend to retain contaminants like heavy metals and PAHs more strongly, which makes their removal by the physical and chemical treatments currently in use more difficult (Dennis et al. 1992). In addition to clay, the organic matter content is also associated

Table 2 Physical-chemical characterization of the experimental soil

Parameter	Value
Thick sandy (%)	18.0
Fine sand (%)	17.0
Silt (%)	3.0
Clay (%)	62.0
pH (in H_2O)	4.3
Al^{3+} ($cmol_c$ dm^{-3})	1.2
Ca^{2+} ($cmol_c$ dm^{-3})	0.1
Mg^{2+} ($cmol_c$ dm^{-3})	0.1
Organic matter (g kg^{-1})	33.2
P (Mehlich-1) (mg dm^{-3})	2.0
K (Mehlich-1) (mg dm^{-3})	29.0
P rem (mg l^{-1})[a]	11.0

[a] Phosphorus remaining in mg l^{-1} obtained by the agitation of 60 mg l^{-1} of P in a solution of $CaCl_2$ 10 mmol l^{-1} with the soil sample in a reaction soil: solution 1:10 for 1 h

with the retention of contaminants (Mulligan et al. 2001).

Procedure for soil contamination

2.5 grams of soil samples were contaminated with Cd^{2+} and phenanthrene. Soil contamination with Cd^{2+} was performed as described by Mulligan et al. (1999). The contamination procedure consisted of agitation of soil samples for 72 h, after the addition of 10 ml per gram of soil of a solution containing 1 mmol l^{-1} of cadmium nitrate tetrahydrate (Cd $(NO_3)_2.4H_2O$). The soil was removed from solution by centrifugation (1350 g, 30 min). Soil samples were then contaminated with phenanthrene dissolved in acetone (1 mg ml^{-1}) (Yang et al. 2006). The contaminated soil, at a final concentration of 200 mg kg^{-1} of phenanthrene, was stirred vigorously for 30 min to promote homogenous distribution of phenanthrene. Soil samples were left to rest for 48 h at 28°C to eliminate the acetone.

Soil washing procedure

The washing agent was prepared by mixing surfactant with iodide, to form a pseudo-single-phase with pH adjusted to 7.0–8.0. Iodide concentrations of 0, 0.168 and 0.336 mol l^{-1} were tested. Distilled water was used as a control.

Soil samples (2.5 g) were saturated with 25 ml of washing solution and the mixture was kept under agitation at 150 rpm for 24 h. The soil sample was removed from the solution by centrifugation at $1350\times g$ for 30 min. To determine the amount of Cd^{2+} removed from the samples, the Cd^{2+} concentration in the supernatant was analyzed by atomic absorption spectrophotometry (AAS) (Shin et al. 2004). The percentage of Cd^{2+} removed was determined with the following equation:

$$[\%\ \text{removed}\ Cd^{2+} = (\text{Amount of}\ Cd^{2+}\text{in the supernatant} / \text{the total amount of soil}\ Cd^{2+}) \times 100]$$

A series of five consecutive batch washes was performed on each soil sample, using new washing solutions each time. At each stage of washing, the solution-soil mixture was agitated for 24 h, followed by centrifugation ($1350\times g$, 30 min) and analysis of the Cd^{2+} in the supernatant.

Analysis of residual phenanthrene

To determine the amount of phenanthrene removed from the samples, the concentration of phenanthrene in the supernatant was analyzed by spectrophotometry at 254 nm. The percentage of phenanthrene removed was calculated based on the content of initial phenanthrene applied to the soil and final content removed after the five stages of washing. Soil samples, without phenanthrene and Cd^{2+}, washed with the same surfactant-ligand solutions, were used as controls.

Statistical analysis

The experiment was conducted using a factorial scheme $[(5 \times 3) + 1]$ completely randomized design, corresponding to five surfactants and three ligand (I^-) concentrations as well as a control (distilled water), with three repetitions. The data collected were submitted to analysis of variance (ANOVA) at 5% probability, followed by comparison of means (Tukey) or regression analysis, when pertinent.

Results and discussion

Cd^{2+} soil removal by surfactants-ligand solutions

Pure biosurfactant solutions were able to remove about 70% of Cd^{2+} present in the soil, an amount significantly higher than obtained with the synthetic surfactant Triton X-100 solution, which removed only 28.7% of the Cd^{2+} (Table 3). The efficiency of Triton X-100 in removing Cd^{2+} in the absence of ligand was low compared to the removal efficiency achieved by the pure biosurfactant solutions (Fig. 1 and Table 3).

Cd^{2+} removal increased significantly with the number of washes and the ligand concentration for most of the surfactants evaluated (Fig. 1). In combination with the largest ligand concentration, the total removal of Cd^{2+} obtained after the five stages of washing reached values of 99.2% (LBBMA 111A), 99.2% (LBBMA 201), 84.4% (LBBMA 155), 74.1%

Table 3 Cd^{2+} removal of a Haplustox soil by surfactants combined with different concentrations of the ligand iodide (I^-)

Surfactant	Ligand (mol l^{-1})	Cd^{2+} initial (mg g^{-1})	Total Cd^{2+} removed (mg g^{-1})	Ligand × Cd^{2+} (α, β, r^2)[c]	Total Cd^{2+} removed (%)
111A[a]	[0]	1.124	0.808 cd		71.9
111A	[0.168]	1.124	0.928 b	$\alpha = 0.914$**	82.6
111A	[0.336]	1.124	1.115 a	$r^2 = 0.98$	99.2
155[a]	[0]	1.124	0.793 cd	$\alpha = 1.178$*	70.6
155	[0.168]	1.124	0.933 b	$\beta = -2.147$°	83.0
155	[0.336]	1.124	0.949 b	$r^2 = 0.79$	84.4
168[a]	[0]	1.124	0.752 cd		66.9
168	[0.168]	1.124	0.793 cd	$\alpha = 0.237$**	70.6
168	[0.336]	1.124	0.832 c	$r^2 = 0.76$	74.0
201[a]	[0]	1.124	0.767 cd	$\alpha = 2.521$**	68.2
201	[0.168]	1.124	1.067 a	$\beta = -4.401$**	94.9
201	[0.336]	1.124	1.116 a	$r^2 = 0.98$	99.2
Triton X-100[b]	[0]	1.124	0.323 f	$\alpha = 2.856$**	28.7
Triton X-100	[0.168]	1.124	0.663 e	$\beta = -4.885$**	59.0
Triton X-100	[0.336]	1.124	0.731 de	$r^2 = 0.99$	65.0
Water	–	1.124	0.162 g		14.4

[a] *LBBMA 111A* Surfactant produced by isolate *Bacillus* sp. LBBMA 111A, *LBBMA 155* Surfactant produced by isolate *Bacillus subtilis* LBBMA 155, *LBBMA 201* Surfactant produced by isolate *Arthrobacter oxydans* LBBMA 201, *LBBMA 168* Surfactant produced by isolate *Flavobacterium* sp. LBBMA 168

[b] *Triton X-100* Synthetic surfactant

[c] Estimated parameters and r^2 of linear (α) and quadratic (α, β) regression models of I^- concentration and Cd^{2+} removed. °, *, ** Significant at 10, 5 and 1% of probability, respectively

Means followed by the same letter, within a column, do not differ by Tukey test, at 5% of probability

(LBBMA 168) and 65.0% (Triton X-100) (Fig. 1 and Table 3).

The mechanism suggested for the increase of removal efficiency of Cd^{2+} by the presence of I^- in the washing solution is the formation of a molecule formed by complexation of the target metal with the ligand, followed by its retention within the surfactant micelle (Umebayashi et al. 1997).

The presence of the ligand ion facilitated the removal of metal ion from the soil, except when using surfactant LBBMA 168, a flavolipid, whose removal capacity was little influenced by the ligand, as the total removal of Cd^{2+} in the absence of the ligand, obtained after the five wash stages reached 66.9% (Fig. 1 and Table 3). The flavolipids are surfactants with the ability to complex cadmium (Bodour et al. 2004).

High efficiency of Cd^{2+} removal, after the five washes was observed for LBBMA 201 (99.2%) and LBBMA 111A (99.2%) surfactants (Fig. 1 and Table 3). The synthetic surfactant Triton X-100 reached removal efficiency values of 59 and 65% when combined with 0.168 and 0.336 mol l^{-1} of I^-, respectively (Fig. 1 and Table 3). The removal of Cd^{2+} by water was less than 15% (Table 3).

When the soil was washed with solution containing Triton X-100, in the presence of 0.0, 0.168 and 0.336 mol l^{-1} of ligand, the total removal of Cd^{2+} was 28.7, 59.0 and 65.0%, respectively, after five washes (Fig. 1 and Table 3). It can be concluded that in the absence of the ligand, Triton X-100 is not effective in removing soil Cd^{2+}. However, in the presence of the ligand, the removal of Cd^{2+} increased significantly (Fig. 1 and Table 3). This effect was also observed by Shin and Barrington (2005). In that work, the Cd^{2+} removal from soil contaminated by Triton X-100 in the absence of iodide ligand was only 10% of Cd^{2+} present in the soil.

Anionic biosurfactants like the rhamnolipids have a high ability to complex with heavy metals, showing

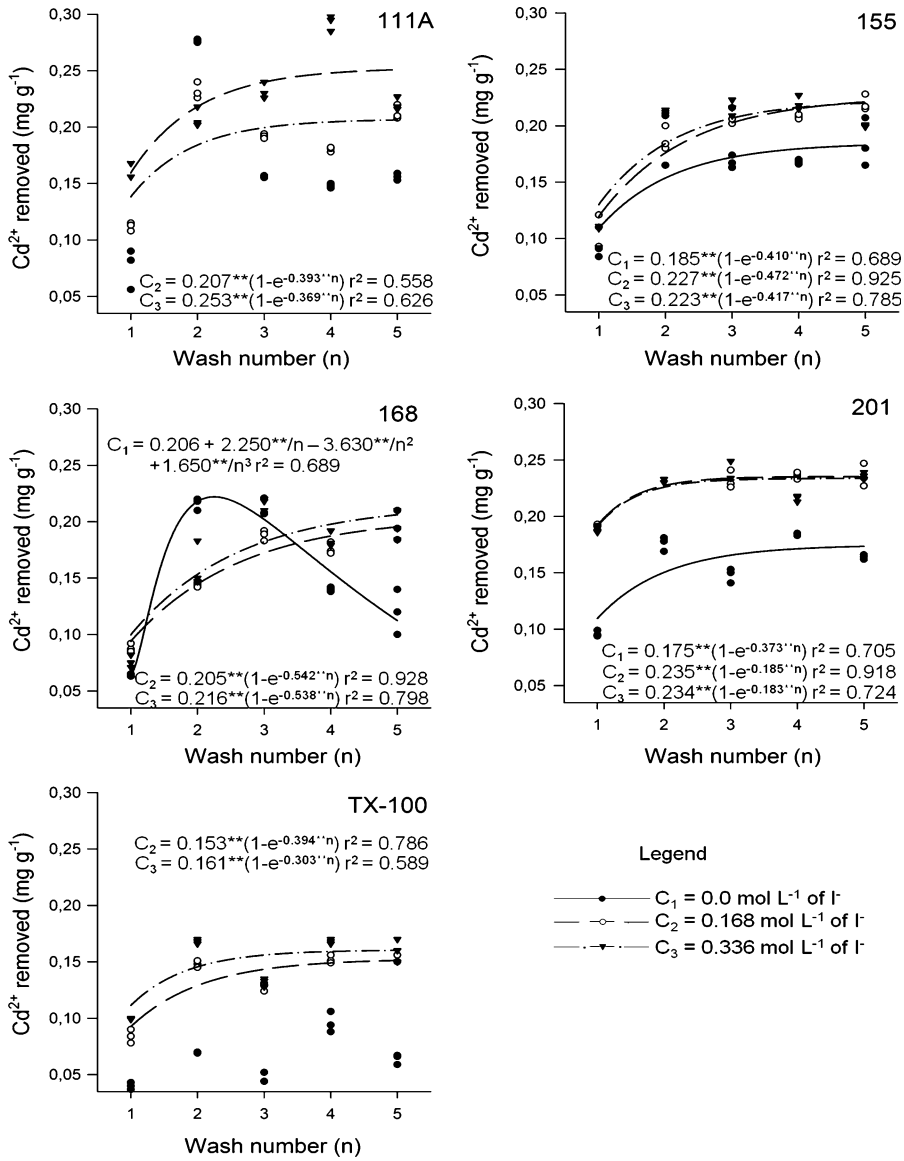

Fig. 1 Effect of successive washings on the removal of Cd^{2+} from soil. **111A** (Surfactant LBBMA 111A), **155** (Surfactant LBBMA 155), **168** (Surfactant LBBMA 168), **201** (Surfactant LBBMA 201), **TX-100** (Surfactant Triton X-100). ** Significant at 1% of probability

a greater affinity towards Cd^{2+} than towards other common soil cations, such as Mg^{2+} and K^{+} (Mulligan 2005). This feature is important for the application of these compounds in heavy metal-contaminated soil washing process because plants and soil microorganisms essential nutrients will be at least partially preserved.

Pure biosurfactant solutions removed between 79 and 87% of phenanthrene present in the soil. These values were significantly higher than the removal by Triton X-100, which facilitated the removal of about 73% of the contaminant (Tables 3 and 4). Most of the phenanthrene (over 92% of the total removed) was removed from the soil in the first stage of washing. The result indicates that in commercial applications a single soil wash with a solution of biosurfactants in concentration equivalent to 2CMC is enough to remove most of the phenanthrene and possibly other organic hydrophobic contaminants.

Addition of the iodide ligand to surfactant solutions did not significantly influence the removal of phenanthrene, except for the Triton X-100 solution (Table 4 and Fig. 2). There was no detectable removal of phenanthrene by water, emphasizing the necessity of

Table 4 Efficiency of removal of phenanthrene, after treating the Haplustox soil with surfactants in the presence/absence of ligand

Surfactant	Ligand (mol l^{-1})	PHE initial (μg g^{-1})	Total PHE removed (μg g^{-1})	Ligand x PHE (α, β, r^2)[c]	Total PHE removed (%)
111A[a]	[0]	200	173.2 abc		86.6
111A	[0.168]	200	174.0 abc	$\alpha = 0.000$ [ns]	87.0
111A	[0.336]	200	174.4 abc	$r^2 = 0.00$	87.2
155[a]	[0]	200	158.4 d		79.2
155	[0.168]	200	162.4 cd	$\alpha = 0.750$**	81.2
155	[0.336]	200	168.8 abcd	$r^2 = 0.89$	84.4
168[a]	[0]	200	164.4 bcd		82.2
168	[0.168]	200	165.6 abcd	$\alpha = 0.015$°	82.8
168	[0.336]	200	166.8 abcd	$r^2 = 0.64$	83.4
201[a]	[0]	200	170.4 abc		85.2
201	[0.168]	200	175.6 ab	$\alpha = 0.043$**	87.8
201	[0.336]	200	176.4 ab	$r^2 = 0.65$	88.2
Triton X-100[b]	[0]	200	146.4 e		73.2
Triton X-100	[0.168]	200	170.8 abc	$\alpha = 0.226$**	85.4
Triton X-100	[0.336]	200	176.8 a	$r^2 = 0.89$	88.4
Water	–	200	0.000 f		0.0

°, ** Significant at 10 and 1% of probability, respectively

[a] *LBBMA 111A* Surfactant produced by isolate *Bacillus* sp. LBBMA 111A, *LBBMA 155* Surfactant produced by isolate *Bacillus subtilis* LBBMA 155, *LBBMA 168* Surfactant produced by isolate *Flavobacterium* sp. LBBMA 168, *LBBMA 201* Surfactant produced by isolate *Arthrobacter oxydans* LBBMA 201

[b] *Triton X-100* Synthetic surfactant

[c] Estimated parameters and r^2 of linear (α) regression model of I^- concentration and phenanthrene removed

Means followed by the same letter, within a column, do not differ by Tukey test at 5% of probability

including surfactants for the remediation of contaminated soils with hydrophobic pollutants.

Efficiency of surfactants-ligand solutions in simultaneous removal of Cd^{2+} and phenanthrene

Phenanthrene was removed with efficiency by solutions containing only biosurfactants at a concentration equivalent to 2CMC (Table 4). The addition of iodide ligand to biosurfactant solutions did not affect their ability to remove phenanthrene. If on the one hand the addition of ligand did not result in greater removal efficiency of the hydrophobic compound, on the other hand it didn't compromise the removal. For removal of Cd^{2+}, and possibly other heavy metals, a high ligand concentration increased the efficiency of the surfactants, especially LBBMA 111A, LBBMA 168 and Triton X-100 (Fig. 1 and Table 3).

Based on the results of this work, it can be concluded that solutions containing a surfactant-ligand combination efficiently remove Cd^{2+} and phenanthrene from contaminated soil. A plausible mechanistic scheme for the removal process involves initial ligand–metal complexation to form ionic species, followed by internalization of the Cd-I^- complex and phenanthrene in the hydrophobic core of the surfactant micelles (Shin et al. 2004). It is proposed that surfactant-ligand solutions are also capable of simultaneously removing other hydrophobic and metallic pollutants from contaminated soil and sediment.

Conclusions

The removal of Cd^{2+} by the surfactant in contaminated soil was significantly improved by the presence of iodide. Surfactant/ligand systems can efficiently

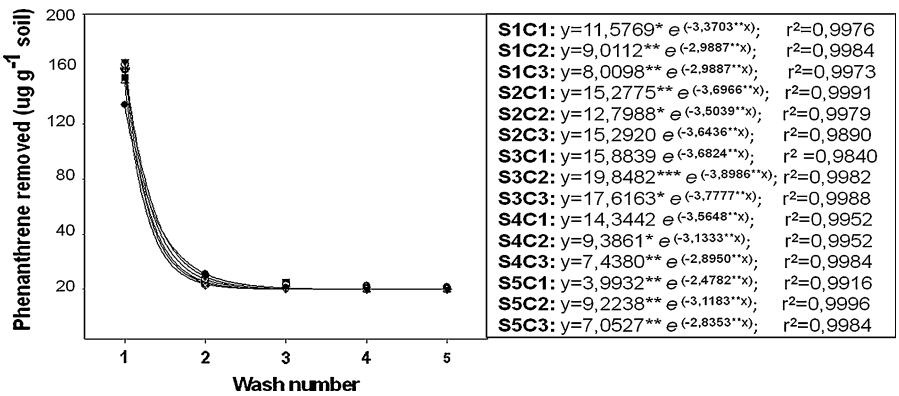

Fig. 2 Effect of successive washings on phenanthrene removal from soil. **S1** (Surfactant LBBMA 111A), **S2** (Surfactant LBBMA 155), **S3** (Surfactant LBBMA 168), **S4** (Surfactant LBBMA 201), **S5** (Surfactant Triton X-100), **C1** (0.0 mol l^{-1} of I^-), **C2** (0.168 mol l^{-1} of I^-), C3 (0.336 mol l^{-1} of I^-). ***,*,** Significant at 10, 5 and 1% of probability, respectively

remove phenanthrene, and this removal is also successful in the presence of the ligand. The increase in the ligand concentration improved the removal of Cd^{2+}, but with a smaller effect on the removal of phenanthrene. Cd^{2+} and phenanthrene can be removed simultaneously from soils by using a surfactant-ligand solution.

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References

Abramovitch RA, Chang-Qing L, Hicks E, Sinard J (2003) In situ remediation of soils contaminated with toxic metal ions using microwave energy. Chemosphere 53:1077–1085

Bodour AA, Miller-maier RM (1998) Application of a modified drop-collapse technique for surfactant quantification and screening of biosurfactant-producing microorganisms. J Microbiol Method 32:273–280

Bodour AA, Guerrero-Barajas C, Jiorle BV, Malcomson ME, Paul AK, Somogyi A, Trinh LN, Bates RB, Miller-Maier RM (2004) Structure and characterization of flavolipids, a novel class of biosurfactants produced by *Flavobacterium* sp. MTN11. Appl Environ Microbiol 70:114–120

Chu W, Kwan CY (2003) Remediation of contaminated soil by a solvent/surfactant system. Chemosphere 53:9–15

Dacera DDM, Babel S (2008) Removal of heavy metals from contaminated sewage sludge using *Aspergillus niger* fermented raw liquid from pineapple wastes. Biores Technol 99:1682–1689

Dennis RM, Dworkin D, Lowe WL, Zukpo AJ (1992) Evaluation of commercially available soil-washing processes for site remediation. J Hazard Ind Was 24:515–525

Fava F, Di Gioia D (1998) Effects of Triton X-100 and quillaya saponin on the ex situ bioremediation of a chronically polychlorobiphenyl-contaminated soil. Appl Microbiol Biotechnol 50:623–630

Fernandes VC, Albergaria JT, Oliva-Teles T, Delerue-Matos C, De Marco P (2009) Dual augmentation for aerobic bioremediation of MTBE and TCE pollution in heavy metal-contaminated soil. Biodegradation 20:375–382

Hendershot WH, Lalande H, Duquette M (1993) Soil sampling and methods of analysis. Lewis Publishers, Boca Raton

Hirner AV, Pestke FM, Busche U, Eckelhoff A (1998) Testing contaminant mobility in soils and waste materials. J Geochem Explor 64:127–132

Ho KC, Hui KCC (2001) Chemical contamination of the east river (Dongjiang) and its implication on sustainable development in the Pearl River Delta. Environ Int 26:303–308

Horowitz S, Gilbert JN, Grilfin WM (1990) Isolation and characterization of a surfactant produced by *Bacillus licheniformis* 86. J Ind Microbiol 6:243–248

Jaffe R, Gardinali PR, Cai Y, Sudburry A, Fernandez A, Hay BJ (2003) Organic compounds and trace metals of anthropogenic origin in sediments from Montego Bay, Jamaica: assessment of sources and distribution pathways. Environ Poll 123:291–299

Lin SC, Chen YC, Lin YM (1998) General approach for the development of high-performance liquid chromatography methods for biosurfactant analysis and purification. J Chromatogr 859:149–159

Mulligan CN (2005) Environmental applications for biosurfactants. Environ Poll 133:183–198

Mulligan CN, Yong RN, Gibbs BF, James S, Bennett HPJ (1999) Metal removal from contaminated soil and sediments by the biosurfactant surfactin. Environ Sci Technol 33:3812–3820

Mulligan CN, Yong RN, Gibbs BF (2001) Heavy metal removal from sediments by biosurfactants. J Hazard Mater 85:111–125

Reddy KR, Chinthamreddy S (2000) Comparison of extractants for removing heavy metals from contaminated clayey soils. Soil Sediment Contam. 9:449–462

Schmelling D, Poster D, Chaychian M, Neta P, Mclaughlin W, Silverman J, Al-Sheikhly M (1998) Application of ionizing radiation to the remediation of materials contaminated with heavy metals and polychlorinated biphenyls. Radiat Phys chem 52:371–377

Shin M, Barrington SF (2005) Effectiveness of the iodine ligand along with two surfactants on desorbing heavy metals from soils. Water Air Soil Poll 161:193–208

Shin M, Umebayashi Y, Kanzaki R, Ishiguro S-I (2000) Formation of copper(II) thiocyanato and cadmium(II) iodo complexes in micelles of nonionic surfactants with varying poly(ethylene oxide) chain lengths. J Colloid Interface Sci 225:112–118

Shin M, Barrington SF, Marshall WD, Kim JW (2004) Simultaneous soil Cd and PCB decontamination using a Surfactant/ligand solution. J Environ Sci Health 39:2783–2798

Soil Survey Staff (1999) Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. Agricultural Handbook 436, 2nd edn. US Department of Agriculture Soil Conservation Service, Washington, DC

Umebayashi Y, Shin M, Ishiguro SI (1997) Thiocyanato and iodo complexation of cadmium (II) ions in micellar solutions of a nonionic surfactant Triton X-100. J Colloid Interface Sci 191:391–397

Vater J, Kablitz B, Wilde C, Franke P, Mehta N, Cameotra SS (2002) Matrix-assisted laser desortion ionization-time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. Appl Environ Microbiol 68:6210–6219

Wasay SA, Barrington S, Tokunaga S (2000) Organic acids for the in situ remediation of soils polluted by heavy metals: soil flushing in columns. Water Air Soil Poll 127:301–314

Weiss P, Riss A, Gschmeidler E, Schentz H (1994) Investigation of heavy metal, PAH, PCB patterns and PCDD/F profiles of soil samples from an industrialized urban area (Linz, upper Austria) with multivariate statistical methods. Chemosphere 29:2223–2236

Yang K, Zhu L, Xing B (2006) Enhanced soil washing of phenanthrene by mixed solutions of TX100 and SDBS. Environ Sci Technol 40:4274–4280

Yeomans JC, Bremner JM (1988) A rapid and precise method for routine determination of organic carbon in soil. Commun Soil Sci Plant Anal 19:1467–1476