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Production of bioemulsifiers by *Amycolatopsis tucumanensis* DSM 45259 and their potential application in remediation technologies for soils contaminated with hexavalent chromium

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HIGHLIGHTS

- *Amycolatopsis tucumanensis* DSM 45259 was able to produce bioemulsifiers.
- The best specific production was detected using glycerol and urea as carbon and nitrogen sources.
- The chemical nature of bioemulsifiers was dependent upon the carbon source.
- Bioemulsifiers were very stable at extreme conditions of pH, temperature, and salt concentration.
- Bioemulsifiers were effective in removing Cr(VI) from soil by washing technologies.

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ABSTRACT

In recent years, increasing interest has been shown in the use of bioemulsifiers as washing agents that can enhance desorption of soil-bound metals. However, high production costs derived from the use of expensive substrates for formulation of the fermentation media represent the main challenge for full, large-scale implementation of bioemulsifiers. This work reports on a first study of bioemulsifier production by the actinobacterium *Amycolatopsis tucumanensis* DSM 45259 using different carbon and nitrogen sources. Preliminary results on the potential use of these compounds as washing agents for soils contaminated with Cu(II) and Cr(VI) are also presented. The best specific production was detected using glycerol and urea as carbon and nitrogen substrates, respectively. However, with all of the substrates used during the batch assay, the bioemulsifiers showed high levels of stability at extreme conditions of pH, temperature, and salt concentration. Under the current assay conditions, the bioemulsifiers were not effective in removing Cu(II) from soil. However, they were able to mediate Cr(VI) recovery, with the removal percentage doubled compared to that seen when using deionized water. These findings appear promising for the development of remediation technologies for hexavalent chromium compounds based upon direct use of these microbial emulsifiers.

1. Introduction

A diversity of microorganisms including bacteria, filamentous fungi, and yeasts are able to produce a wide range of amphipathic compounds that exhibit surface activities at interfaces, such as bioemulsifiers [1–3]. Unlike their synthetically produced counterparts, emulsifiers of natural origin are biodegradable and have reduced toxicity, which is in agreement with the concept of environmental sustainability [4]. In addition, they can remain effective even at extreme conditions of pH, temperature, and salinity. These properties increase their scope of applicability in a diverse range of biotechnological areas.

In the field of bioremediation, the application of bioemulsifiers as natural alternatives to synthetic production is an efficient strategy for removing hydrocarbons from contaminated soils and sediments [5–7]. However, remediation of heavy metal contamination brings up several unique challenges, since these metals cannot be degraded to innocuous products [8]. In addition, metals

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tend to be strongly absorbed on the matrix of soils and sediments, which limits their solubility and hinders their subsequent removal. Effective methods for increasing metal desorption in soils and sediments involve washing technologies assisted by surface-active compounds such as bioemulsifiers [9–11]. The use of bioemulsifiers in a washing technology can also enhance the bioavailability of heavy metals and other pollutants for microbial uptake [12]. Despite their potential applications in multiple biotechnological areas, the commercial availability of bioemulsifiers is currently limited. This is mainly due to economic obstacles to their sustainable production at industrial levels. The production of new biomolecules and strains could therefore be key for overcoming these limitations and challenges, especially if inexpensive substrates can be used. It is important to remark that substrates represent 10–30% of total production costs [13].

In previous studies, the use of the homeostasis system of the actinobacterium *Amycolatopsis tucumanensis* DSM 45259 as a strategy to reduce Cu(II) and Cr(VI) contamination in a diverse set of media has been proposed [14]. In the present work, a first study on the production and partial characterization of bioemulsifiers obtained from this strain using different carbon and nitrogen sources is presented. The potential use of these bioemulsifiers for washing soils contaminated with Cu(II) or Cr(VI) is also analyzed.

2. Experimental

2.1. Microorganism, maintenance and culture conditions

A. tucumanensis DSM 45259, from the culture collection at PROIMI (Pilot Plant of Microbiological Industrial Processes, Tucumán, Argentina) was used in this work. This strain was maintained on Starch-Casein agar slants (SC agar) containing (g/L): starch, 10.0; casein, 1.0; K_2HPO_4, 0.5; and agar, 12.0.

A. tucumanensis DSM 45259 spore standardized suspensions harvested from SC agar were inoculated in liquid minimal medium (MM) formulated by Amoroso et al. [15], and modified for this study to contain (in g/L): glucose as carbon source (C), 10.0; L-asparagine as nitrogen source (N), 0.5; K_2HPO_4, 1.5; $MgCl_2{\cdot}7H_2O$, 0.20; and $FeSO_4{\cdot}7H_2O$, 0.01. The bioemulsifier production detected in this medium was used as a reference. To evaluate the effects of the carbon and nitrogen sources on bioemulsifier production, glucose was replaced by glycerol or sodium carbonate, while L-asparagine was replaced by urea or ammonium nitrate, at the same concentrations as used for the initial sources. Based upon these experiments, possible combinations of the selected carbon and nitrogen sources were evaluated. The cultures were incubated in Erlenmeyer flasks on an orbital shaker (170 rpm) at 30 °C for 120 h, with previous adjustment to an initial pH of 7.0.

2.2. Determination of cell growth

To estimate the microbial biomass, the samples were centrifuged at 10,000 × *g* for 15 min at 4 °C, and the cells were washed twice with bi-distilled water. Dry weight was determined using aluminum foil cups dried at 80 °C to constant weight. The supernatants were stored at 4 °C for subsequent analysis.

2.3. Bioemulsifier production

Bioemulsifier production was expressed as the specific emulsification index (q_{EI}), which is estimated as the emulsification index (EI) of the culture supernatant per g of biomass. To calculate the EI, equal volumes of the culture supernatant and a hydrocarbon such as kerosene were mixed together, with this mixture then vortexed for 2 min and left to settle. The EI was calculated as the percentage obtained by dividing the height of the emulsified layer (mm) by the total height of the liquid column (mm) [16]. Emulsion stability was determined according to Bosch et al. [17], who defined an emulsion as stable when the EI is 50% or higher after being left to settle for 24 h.

Table 1
Physicochemical characteristics of the natural garden soil used.

Parameters	
pH[a]	7.7
EC, dS/m[b]	0.7
Calcium carbonate, %[c]	1.0
Textural class[d]	Loam
Organic carbon, %[e]	2.7
Phosphorous, ppm[f]	16.7
Sodium exchange capacity, Cmolc/kg[g]	0.4
Potassium exchange capacity, Cmolc/kg[g]	1.2
Calcium exchange capacity, Cmolc/kg[g]	–
Magnesium exchange capacity, Cmolc/kg[g]	–
CEC, Cmolc/kg[g]	18.8

[a] Soil to distilled water ratio of 1:2.5.
[b] Electrical conductivity method.
[c] Gasometric method.
[d] Capillary method.
[e] Walkley and Black method.
[f] Bray and Kurtz I.
[g] Cation exchange capacity determined by ammonium-sodium acetate method.

2.4. Nature and partial characterization of the bioemulsifier

The bioemulsifiers produced were extracted from the culture supernatants by precipitation with acetone (1:1, v/v), at 4 °C for 24 h [18]. The precipitates were recovered by filtration through a 0.45 μm membrane, and then dissolved in distilled water. To estimate the presumptive nature of the partially purified bioemulsifiers, the aqueous extracts were subjected to hydrolytic treatments using proteinase K (30 U/mg, at 37 °C for 4 h), Lipolase 100 L from *Thermomyces lanuginosus* (10 U/mg, at 37 °C for 2 h), and HCl at 100 °C. The thermal resistance of the bioemulsifiers was tested by incubating extracts at a range of temperatures from 37–100 °C, for 1 h at each temperature. Stability at different pH values was evaluated by incubating the extracts at 37 °C for 1 h in 1 M buffers of citric acid-Na_2HPO_3 (pH 3.0, 4.0, and 5.0), Na_2HPO_3-NaH_2PO_3 (pH 6.0, 7.0, and 8.0), and Na_2CO_3-$NaHCO_3$ (pH 9.0 and 10.0). Resistance to salinity was determined by exposure of the emulsifier extracts to various concentrations of NaCl (5–20%, w/v) at 37 °C, for 1 h at each concentration. After each treatment the residual EI was calculated again, with the extracts without treatment used as controls representing 100% emulsifying activity.

2.5. Removal of heavy metals from soils

2.5.1. Analysis and preparation of the soils

The physicochemical characteristics of a natural soil collected from a garden area were determined in the soils laboratory at the Colombres Obispo Agroindustrial Experimental Station (Table 1). After this analysis, the soil samples were artificially contaminated with Cu(II) or Cr(VI), added as $CuSO_4{\cdot}5H_2O$ and $K_2Cr_2O_7$ from stock solutions, respectively, to a final concentration of 200 mg/kg of soil. Soil samples were dried at 105 °C to a constant weight, and then subjected to the washing experiments.

2.5.2. Soil washing experiments

Soil washing experiments were performed according to Mulligan et al. [19] with minimal modifications. First, 2.0 g of the

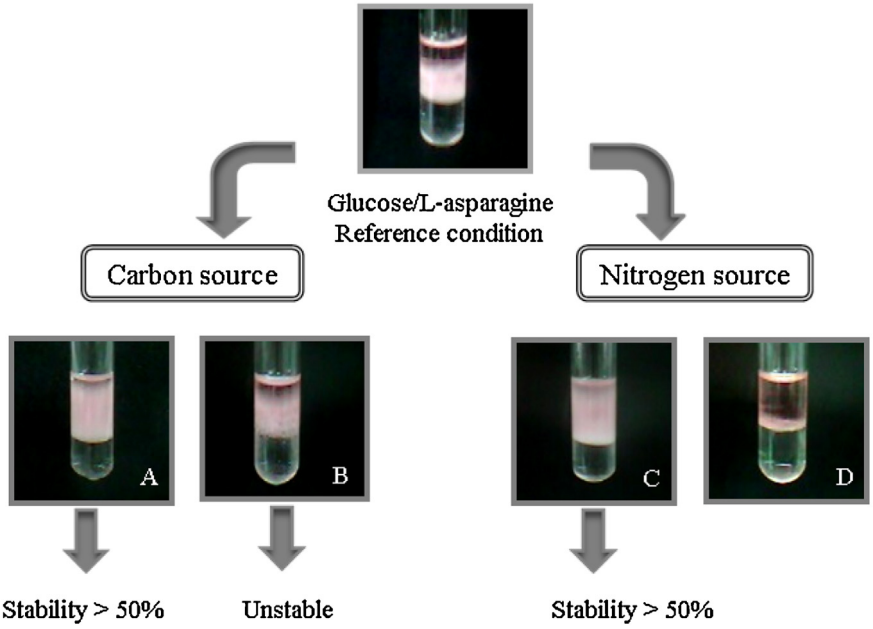

Fig. 1. Bioemulsifier production by *Amycolatopsis tucumanensis* DSM 45259 when varying either the carbon sources: (A) glycerol/L-asparagine and (B) sodium carbonate/L-asparagine; or the nitrogen sources: (C) glucose/urea and (D) glucose/ammonium nitrate.

contaminated soil samples were placed in glass bottles for washing with 10.0 ml of the aqueous solutions of the partially purified bioemulsifiers. Deionized water (H_2O_d) was used as control in order to account for removal of contaminants by physical mixing. In all cases, the EI of the bioemulsifier solution was first adjusted to 60%. The washing was performed by shaking at 30 °C for 12–24 h. The soil samples were then centrifuged at 10,000 × *g* for 10 min in order to obtain the supernatants. The concentration of each metal was then analyzed using atomic absorption spectrometry, with the removal percentage expressed based upon each metal's initial concentration. The Cr(VI) concentration was measured using a specific colorimetric reagent (1,5-diphenylcarbazide), dissolved in acetone to a final concentration of 0.05% (APHA, 1992). Absorbance was measured at 540 nm, and Cr(VI) concentration was calculated using a standard curve prepared using a series of Cr(VI) dilutions (1–25 mg/L).

2.6. Statistical design and analysis

Statistical design and analysis were performed using Info Stat (version 2004) and Minitab (version 14; Minitab) software for Windows. Statistical significance values for the means were evaluated using one-way analysis of variance. Differences were accepted as significant when $p < 0.05$. The main effects and interactions of some experimental variables in terms of growth, bioemulsifier production, and soil washing efficiency were evaluated using full factorial designs. The experimental variables evaluated are presented in Tables 2 and 5, which show the two alternative options tested for each factor. All assays were performed in triplicate with results presented as the mean value ± standard deviation.

Table 2
Factors and their alternative options for growth and bioemulsifier production experiments.

Factor	Option 1	Option 2
Carbon source (C)	Glucose	Glycerol
Nitrogen source (N)	L-asparagine	Urea

3. Results

3.1. Effects of carbon and nitrogen sources on bioemulsifier production

The ability of *A. tucumanensis* DSM 45259 to produce bioemulsifiers from different carbon and nitrogen sources was first evaluated (Fig. 1). Significant bioemulsifier production was detected when glucose (the reference condition) or glycerol (Fig. 1A) were used as the carbon source, with the emulsions formed being very stable (stability >50%). However, with the use of an inorganic carbon source such as sodium carbonate, the results were completely different. Bioemulsifier synthesized from sodium carbonate formed an unstable emulsion with kerosene, with this emulsion practically disappearing after being left to settle for 24 h (Fig. 1B). In regard to cell growth, biomass concentration was drastically reduced when *A. tucumanensis* DSM 45259 was cultivated on sodium carbonate (data not shown). Based upon these results, glucose and glycerol were selected to conduct the subsequent production studies.

The effect of the nitrogen source on bioemulsifier production was also evaluated. We note that the use of L-asparagine (the reference condition) or urea (Fig. 1C) was accompanied by effective bioemulsifier production, with emulsions showing high stability (up to 50%) after settling for 24 h. However, no significant production was detected when the strain was cultivated on an inorganic nitrogen source such as ammonium nitrate (Fig. 1D). Based upon these experiments, the use of ammonium nitrate as a nitrogen substrate to support emulsifier biosynthesis from *A. tucumanensis* DSM 45259 was discarded for future experiments.

3.2. Growth and bioemulsifier production kinetics

Following upon our experiments above, the growth and bioemulsifier production kinetics were evaluated for *A. tucumanensis* DSM 45259 when using combinations of the selected carbon (C) and nitrogen sources (N). The independent variables and the options tested are presented in Table 2.

As seen in Fig. 2, both the biomass concentrations and the q_{EI} values were dependent upon the carbon and nitrogen source used

Table 3
Estimated effects for biomass and q_{EI} determined after 48 h of cultivation.

Term	Biomass (g/L)			q_{EI} (EI%/g biomass)		
	Effects	*T*-values	*p*-values	Effects	*T*-values	*p*-values
Carbon source (C)	−0.302	−3.74	0.020	16.900[a]	4.74	0.009
Nitrogen source (N)	−0.232	−2.88	0.045	20.200[a]	5.67	0.005
CN	−0.232	−2.88	0.045	14.950[a]	4.20	0.014
R-Sq $_{(adj)}$ (%)	79.75			90.83		

[a] Effects relevant for specific bioemulsifier production.

during fermentation. In all cases, the maximum q_{EI} values were detected at 48 h of cultivation (exponential growth phase). It is important to mention that the EI of the supernatants remained constant after 48 h of cultivation (data not shown). Therefore, the decrease in the q_{EI} can be explained by the progressive increase in the biomass. In comparison to the reference condition (Fig. 2A), modifying one factor at a time by either replacing glucose with glycerol (Fig. 2B) or L-asparagine with urea (Fig. 2C) only increased the q_{EI} by about 5–13% at 48 h of cultivation. Furthermore, bacterial growth was not significantly affected during this same time period (Fig. 2B and C). However, the highest bioemulsifier yield was found with simultaneous replacement of glucose and L-asparagine with glycerol and urea, with the q_{EI} increasing by about 95% compared to the reference condition (Fig. 2D). Based upon an estimated effects analyzes of the C and N variables (Table 3), biomass concentration, and to an even greater degree the q_{EI}, were found to be significantly affected by both variables (C and N) as well as by interaction between them (CN). In summary, simultaneous use of glycerol and urea as the carbon and nitrogen substrates, respectively, had the most relevant positive effect on specific bioemulsifier production. Finally, the *R*-Sq$_{(adj)}$ values seen in Table 3 suggest that the factorial model applied explains about 80% of the variability in the measured parameters.

3.3. Nature of the bioemulsifiers and stability studies

The next step was to estimate the presumptive nature of the bioemulsifiers produced using different carbon and nitrogen sources. The emulsifying abilities of the extracts were therefore qualitatively evaluated after being subjected to different hydrolytic treatments, using kerosene as the substrate (Table 4). In all cases, emulsifying ability remained virtually constant after treatment with proteinase K, suggesting that no protein nature was involved in the structure of the emulsifier compounds. Similarly, for those conditions where glycerol was used as the carbon source, the extracts retained 100% of their emulsifying ability after being treated with Lipolase 100 L from *T. lanuginosus*. However, for *A. tucumanensis* DSM 45259 cultivated using glucose, lipolytic action significantly decreased the emulsifying ability of the extracts compared to the untreated controls. This response could be compatible with the presence of lipids in the bioemulsifiers synthesized on glucose. Finally, after acid hydrolysis at 100 °C, no emulsifying ability was detected in the extracts, indicating the presence of sugars in

Table 4
Effects of hydrolytic treatments on the emulsifying ability of the extracts.

Culture condition	Hydrolytic treatments		
	Proteinase K	Lipolase 100 L	Acid hydrolysis
Glucose/L-asparagine	–	+	+++
Glycerol/L-asparagine	–	–	+++
Glucose/urea	–	+	+++
Glycerol/urea	–	–	+++

The "+ and −" notation is used to represent "presence or absence" of effects from the hydrolytic treatments on the emulsifying ability of the extracts.

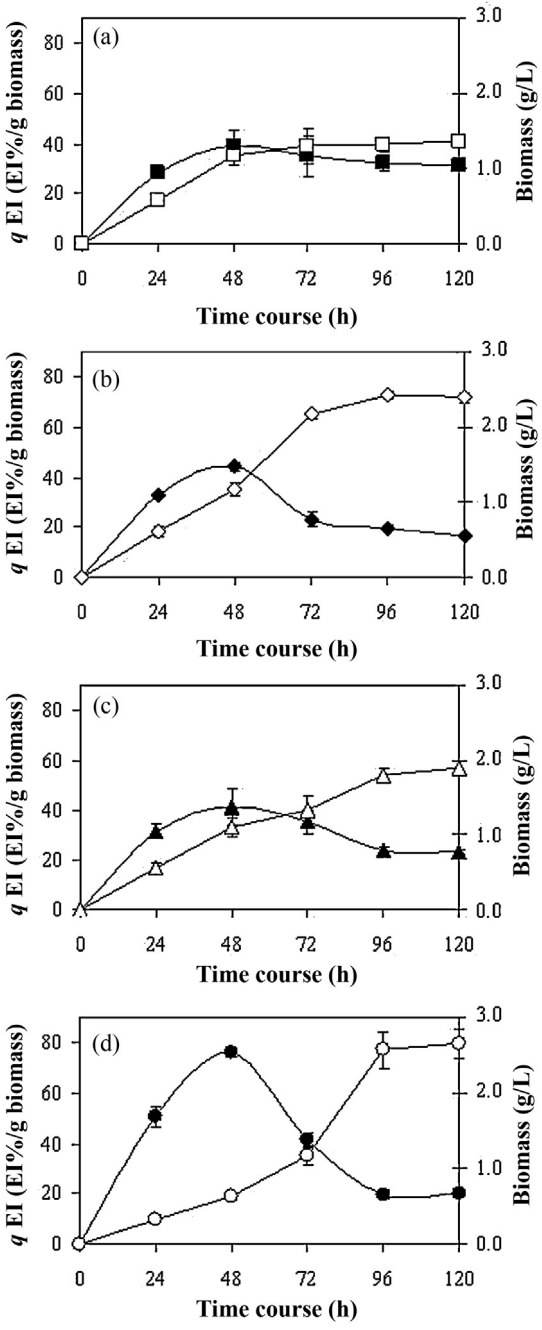

Fig. 2. *Amycolatopsis tucumanensis* DSM 45259 biomass (open symbols) and q_{EI} (solid symbols) during cultivation at 30 °C, with the selected carbon and nitrogen sources as: (A) glucose/L-asparagine (reference condition), (B) glycerol/L-asparagine, (C) glucose/urea, and (D) glycerol/urea. Error bars represent the standard deviation calculated from three independent experiments.

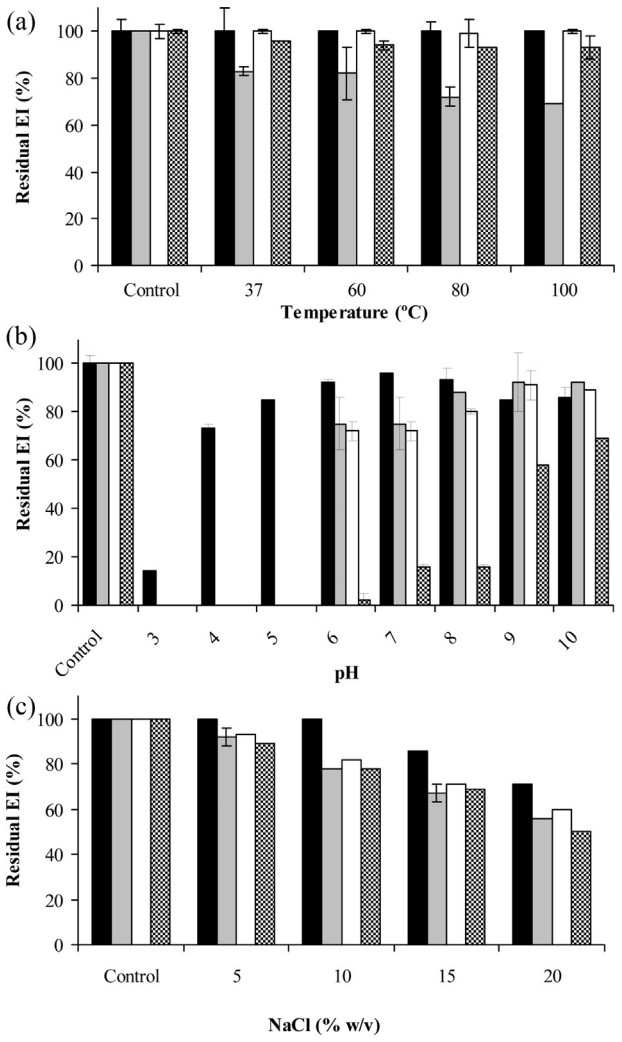

Fig. 3. The effects of temperature (A), pH (B), and NaCl concentration (C) on the residual emulsification index for the extracts of *Amycolatopsis tucumanensis* DSM 45259 obtained using different carbon and nitrogen sources: glucose/L-asparagine (reference condition) (■), glycerol/L-asparagine (▨), glucose/urea (□), and glycerol/urea (▩). The controls correspond to the 100% emulsifying activity of the extracts without treatments. Error bars represent the standard deviation calculated from three independent experiments.

the structure of these compounds. This preliminary study suggests that the bioemulsifiers produced on glucose could be lipopolysaccharides while those produced on glycerol may be mainly based on polysaccharide. However, further studies will be required in order to fully elucidate the nature of these compounds.

In order to assess the potential for the *A. tucumanenisis* DSM 45259 bioemulsifiers to be applied in a variety of fields, the effects of temperature, pH, and NaCl concentration on the residual emulsifying activity were quantitatively determined (Fig. 3). Our results showed that the stability of the bioemulsifiers produced was dependent upon the carbon source used during the batch assay, and to a lesser degree upon the nitrogen source. Under conditions where glucose was used as the carbon source, residual EI remained constant, even after heating at 100 °C for 1 h (Fig. 3A). However, the thermo-stability of the compounds synthesized from glycerol showed a slight decrease in emulsifying ability above 80 °C (Fig. 3A). The *A. tucumanensis* DSM 45259 bioemulsifiers were found to be more sensitive to changes in pH and NaCl concentration than to temperature. While the extract obtained under the reference conditions was active within the full pH range tested (3–10), in the

Table 5
Factors and their alternative options for soil washing experiments using *Amycolatopsis tucumanensis* DSM 45259 bioemulsifiers.

Factor	Option 1	Option 2
Carbon source (C)	Glucose	Glycerol
Nitrogen source (N)	L-asparagine	Urea
Contaminant metal (M)	Cu(II)	Cr(VI)

extracts produced under the other conditions no residual emulsifying activity could be detected in the acidic pH range (3–5). In all cases, optimal values were reached at pH 9–10 (Fig. 3B). In general terms, the bioemulsifiers were very stable across the entire range of NaCl concentrations tested (5–20%, w/v), with those synthesized on glucose found to be the most salt-tolerant (Fig. 3C). It is important to point out that extract produced under the reference condition retained about 70% of its emulsifying ability even at 20% NaCl.

3.4. Washing of soils contaminated with heavy metals

To evaluate the applicability of the *A. tucumanensis* DSM 45259 bioemulsifiers for soil washing technologies, a preliminary study was performed on the efficacy of these compounds in removing Cu(II) and Cr(VI) from artificially contaminated soils. Following Mulligan et al. [19], H_2O_d was used as a control. Table 5 shows the carbon sources (C) and nitrogen sources (N) used for production of the bioemulsifiers, as well as of the type of contaminant metal (M) present in the soil subjected to washing.

Under the assayed conditions, no significant Cu(II) removal could be detected after 12 h of washing. This was true for both the H_2O_d control and for the bioemulsifier solutions, with a removal percentage of less than 2.5% seen in the two cases (Fig. 4). However, the *A. tucumanensis* DSM 45259 bioemulsifiers were found to be more effective in mediating Cr(VI) recovery from soil, with removal percentages doubling in comparison to the H_2O_d (Fig. 4). It is important to point out that increasing the washing time from 12 h to 24 h did not improve the metal removal levels. We were also able to verify that all of the Cr removed during the washing experiments remained in its hexavalent state.

According to the estimated effects analysis of the C, N, and M variables (Table 6), the contaminant metal (M) was the most relevant variable in terms of washing efficiency. The carbon sources (C) and nitrogen sources (N) used for the bioemulsifier production, as well as the interaction effects between them (CN), had only a weak effect on the metal recovery. Finally, interactions between the carbon or nitrogen sources and the contaminant metal (CM and

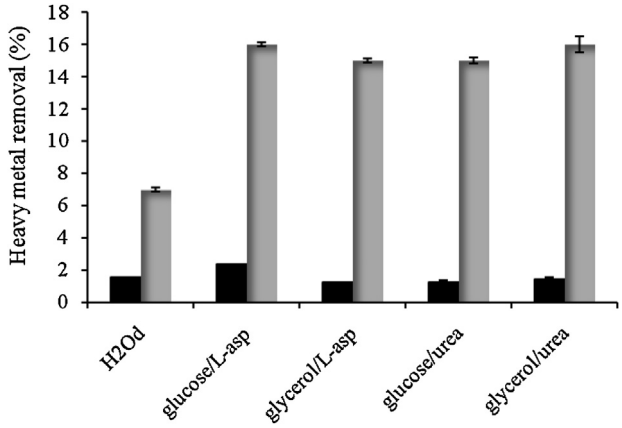

Fig. 4. Removal of Cu(II) (■) and Cr(VI) (□) from artificially contaminated soils, using either H_2O_d as control or bioemulsifier solutions produced by *Amycolatopsis tucumanensis* DSM 45259 with different carbon and nitrogen sources.

Table 6
Estimated effects for metal removal efficiency after 12 h of washing.

Term	Heavy metal removal (%)		
	Effects	*T*-values	*p*-values
Carbon source (C)	−0.241	−2.35	0.047
Nitrogen source (N)	−0.466	−4.54	0.002
Contaminant metal (M)	13.874[a]	135.04	0.000
CN	0.726	7.07	0.000
CM	0.151	1.47	0.179
NM	0.001	0.01	0.991
CNM	0.099	0.96	0.365
$R\text{-}Sq_{(adj)}$ (%)	99.92		

[a] Effect most relevant for soil washing efficiency.

NM) did not have a significant impact on the results of the washing process, nor did the interactions among all three factors (CNM) (Table 6). Based upon the $R\text{-}Sq_{(adj)}$ values (Table 6), it was concluded that the factorial model applied largely explains the variability in the data.

4. Discussion

The main limitations and challenges in the large-scale use of bioemulsifiers are mostly related to their complexity and high production costs [20]. However, microorganisms can use different metabolic pathways to synthesize surface-active compounds. These metabolic pathways are, among other factors, substrate dependent. Microbial production of surface-active compounds using low-cost substrates could therefore represent an important advance in obtaining products of biotechnological interest at the industrial level [21]. While on a worldwide basis biofuels have emerged as promising substitutes for petroleum-based fuels, there is also a great need for the proper management and utilization of the byproducts derived from these bioprocesses. Waste products derived from the production and purification of biodiesel such as crude glycerol could be effectively used as feedstocks for the synthesis of other value-added products [22]. In fact, we have demonstrated the feasibility of using glycerol to support the growth and production of bioemulsifiers by *A. tucumanensis* DSM 45259, which will contribute to the viability of an economical production process. Similarly, de Souza Monteiro et al. [23] have reported on effective production of bioemulsifiers by *Trichosporon mycotoxinivorans* CLA2 using crude glycerol and fatty acids methyl esters derived from biodiesel synthesis as a carbon source. The studies reported here also suggest that replacement of L-asparagine by urea as a nitrogen source may represent a viable strategy for reducing the cost of production. Similar results regarding the production of biosurfactants and bioemulsifiers by other microorganisms using urea as a nitrogen substrate have also been reported by other authors [2,24].

The chemical nature of a bioemulsifier depends upon the type of producer microorganism as well as the production conditions [3]. Along these lines, the preliminary studies presented here suggest that the bioemulsifiers produced by *A. tucumanensis* DSM 45259 may be chemically different depending upon the carbon source used during their biosynthesis. Furthermore, the functional properties of a bioemulsifier depend in part upon their chemical nature, and this largely determinates their applicability in several fields. We have therefore tested the stability of the bioemulsifiers produced in the present study against extreme conditions of pH, temperature, and salt concentration. In comparison to the synthetic sodium dodecyl sulphate surfactant, which shows a significant loss of emulsifying activity beginning at 70 °C [25], the *A. tucumanensis* DSM 45259 bioemulsifiers were quite stable up until 100 °C. This thermal stability could increase their scope of application for removal of heavy metals and other pollutants, including under conditions where high temperatures prevail. Under our assay conditions, high emulsifying activity was also detected at a wide range of pH values, although with higher stability seen at alkaline pH levels than under acidic conditions. However, it is known that different bioemulsifiers can have different pH optima. For example, Luna-Velazco et al. [26] observed that a *Penicillium* sp. bioemulsifier was most active at pH 3–4, with a slight decrease in emulsifying activity at pH 9. Kokare et al. [27], on the other hand, reported on a gradual decrease in stability for *Streptomyces* sp. S1 bioemulsifier as pH was increased from 7 to 9. We also noted a high tolerance level of the *A. tucumanensis* DSM 45259 bioemulsifiers under conditions of extreme salinity. In contrast with the biosurfactant from the marine actinobacterium *Nocardiopsis* sp. B4, which loses about 50% of its emulsifying ability at NaCl concentrations higher than 8% [28], extracts of *A. tucumanensis* DSM 45259 largely retained their emulsifying ability even at 20% NaCl. This salt tolerance could increase the scope of application for these biomolecules, even in conditions where high salt concentrations prevail.

A diverse set of technologies involving the use of the homeostasis systems of environmental microorganisms can be successfully applied to soils, sediments, and waste streams in order to remove heavy metals. However, direct application of microbial products, rather than microorganisms, could bring unquestionable advantages because producer microorganisms would not need to have survival ability in heavy metal-contaminated environments [3]. Biological compounds such as bioemulsifiers can replace synthetic compounds in washing water to assist in the solubilization, dispersal, and desorption of heavy metals in soils and sediments. Under our assay conditions, *A. tucumanensis* DSM 45259 bioemulsifiers were not found to be effective in removing Cu(II) from soil, but they did appear to be appropriate for mediating Cr(VI) recovery. Other studies have also led to similar conclusions, where the effectiveness of bioemulsifiers in binding and removing metals from soils and sediments has varied depending upon the metal itself [9,29]. Metals are involved in a series of complex chemical interactions with the components of the soil [30]. The presence of organic and inorganic ligands has a significant impact on the sorption and desorption processes for the metals in the soil, and these can differ greatly depending upon whether the metals are present as anions or cations. For example, González Hueca et al. [31] found that Cr (in its cationic form), Cu, and Pb are more strongly retained by soil, probably by specific adsorption. Based upon this background, in the present study the presence of carbonates and the high concentration of organic matter in the loam soil used could be responsible for the strong retention of Cu(II) seen. In contrast, the hexavalent chromium present in the soil as an oxyanion had more mobility, which was increased by the presence of the bioemulsifiers. In addition, it must be considered that washing technologies work best in sand and gravel soils rather than in fine-grain soils such as the one used in the present study [32].

Unlike other studies that have found a strong link between the types of substrates used for synthesis of bioemulsifiers and the performance of the bioemulsifiers in removing metals from soil [10], in the present study almost no relation was observed between these variables. However, taking into consideration the cost-benefit balance in terms of supporting an economical production process, glycerol and urea would appear to be more appropriate substrates than glucose and L-asparagine. We also found that increasing the washing time did not improve Cu(II) and Cr(VI) recovery from the soil. This could indicate saturation of the binding sites for the metals on the bioemulsifiers, since it is assumed that high concentrations of surface active compounds are required to mediate effective metal removal [29,33]. In fact, the continual addition of new doses of surface-active compounds to a washing solution is required in order to maximize the recovery of pollutants [4].

Microbial and chemical remediation processes for chromium compounds commonly involve an initial reduction of Cr(VI) to Cr(III). For example, Gnanamania et al. [34] have hypothesized that Cr(VI) remediation carried out by marine *Bacillus* sp. MTCC 5514 occurs through a two-step process. The first step could involve the extracellular enzymatic reduction of the hexavalent species to Cr(III), while the second could involve entrapment of the Cr(III) by a biosurfactant produced by the strain. Conventional chemical processes used for chromium detoxification also involve an initial reduction of Cr(VI) by reductant agents, then subsequent precipitation of the less soluble Cr(III) by pH adjustment. Taking into account the fact that the environmental impact of the hexavalent chromium species is greater than that of the lower oxidation state [35], our studies appear promising in terms of the use of *A. tucumanensis* DSM 45259 bioemulsifiers for direct Cr(VI) recovery without prior reduction. However, further studies are needed in order to optimize metal removal from soil during the washing process.

5. Conclusions

Although the commercial availability of bioemulsifiers is currently limited, their viability in remediation technologies is an established fact, either by ex situ addition or in situ microbial production. Since cost of production is one of the key factors in the development of any biotechnological process, the studies reported here appear to be promising in terms of supporting economical production of bioemulsifiers with the potential for remediation of Cr(VI)-contaminated soils. These are the first advances achieved by our research group in relation to the direct application of microbial products to heavy metal remediation strategies. However, further studies are currently in progress to elucidate the chemical structure of the bioemulsifiers produced, as well as to optimize the process to increase Cr(VI) removal.

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