Development of low-cost culture media for effective biosurfactant production

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INTRODUCTION

Surfactants are chemical compounds widely used in many of the everyday products we use. An increased environmental awareness has led to a great interest in renewable-based, biodegradable and more environmentally friendly surfactants. Biosurfactants exhibit a similar or better performance and have a low impact on the environment comparing with chemical surfactants, due to their low toxicity and high biodegradability. Also, they present high selectivity, low critical micelle concentrations (cmc) and effectiveness at extreme temperatures, pHs and salinities. However, their use depends on whether they can be produced economically at large-scale. The culture medium can account for up to 30-50% of their overall production costs. In that sense, the replacement of expensive synthetic media by inexpensive agro-industrial wastes and by-products, such as corn steep liquor (CSL) and molasses can contribute to reduce their production costs and increase their competitiveness.

AIM: Optimize biosurfactant production by *Pseudomonas aeruginosa* and *Bacillus subtilis* strains using low-cost substrates.

METHODS

P. aeruginosa #112 and *B. subtilis* #573 were isolated from a crude oil sample[1]. Biosurfactant production by these isolates was studied in flasks using agro-industrial by-products (CSL, molasses) obtained from local industries. The surface tension (ST) was measured using the Ring method. The emulsifying activity (E_{24}) was determined with *n*-hexadecane. The biosurfactants were recovered through adsorption chromatography using the polystyrene resin (Amberlite XAD-2) for *P. aeruginosa* #112, and through acidic precipitation for *B. subtilis* #573. The biosurfactants were characterized by ESI-MS, ^{1}H NMR and MALDI-TOF. Oil recovery assays were performed using artificially contaminated sand containing 10% (w/w) of crude oil.

RESULTS

The highest biosurfactant production by *P. aeruginosa* #112 was obtained using a culture medium containing 10% (v/v) of CSL and 10% (w/v) of molasses (medium CSLM). The estimated **price** of this culture medium is **0.024 €/L** [2].

Table 1. Surface tension values [ST_o, ST (mN/m)], emulsifying index [E_{24} (%)], biosurfactant yield (g/L) and cmc (mg/L) obtained with *P. aeruginosa* #112 grown in CSLM medium at 37ºC and 180 rpm for 144 h.

ST_o (mN/m)	ST (mN/m)	E_{24} (%)	[BS] (g/L)	cmc (mg/L)
50.0 ± 0.5	31.6 ± 0.7	62.0 ± 0.0	3.2 ± 0.2	50.0

These biosurfactants were characterized as a mixture of eight different rhamnolipids, being the most abundant the mono-rhamnolipid Rha-C_{10}-C_{10}.

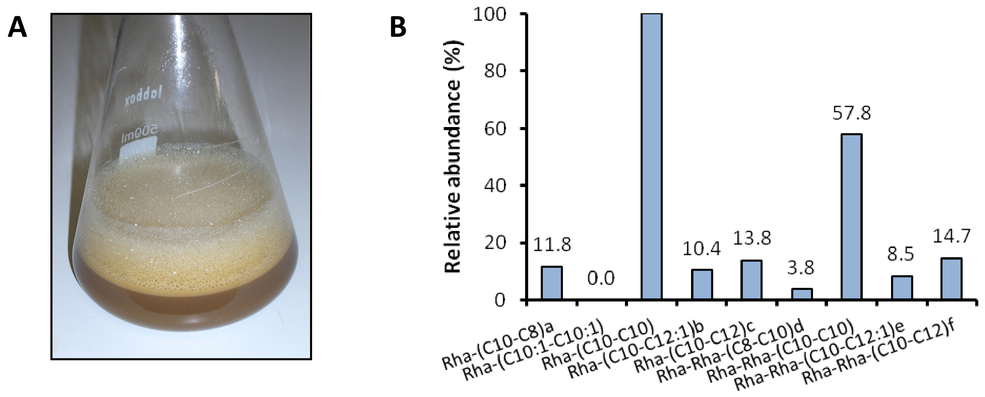

Figure 1. A: *P. aeruginosa* #112 grown in CSLM medium after 144 h. **B:** Relative abundance of the $[M+Na]^+$ ions of the rhamnolipid congeners observed in the ESI–MS spectra obtained in the ESI-Q-TOF mass spectrometer.

The best results with *B. subtilis* #573 were obtained using a culture medium containing 10% (v/v) of CSL. When this medium was supplemented with the optimum combination of metals ($FeSO_4$ 2.0 mM; $MgSO_4$ 0.8 mM; $MnSO_4$ 0.2 mM) a considerable increase in biosurfactant production was achieved. The estimated **price** of this culture medium is **0.004 €/L** [3].

Table 2. Surface tension values [ST_o, ST (mN/m)], emulsifying indexes [E_{24} (%)], biosurfactant yields (g/L) and cmcs (mg/L) obtained with *B. subtilis* #573 grown in CSL and CSL$^+$ media at 37ºC and 200 rpm.

Culture medium	Time (h)	ST_o (mN/m)	ST (mN/m)	E_{24} (%)	[BS] (g/L)	cmc (mg/L)
CSL	48	52.8 ± 0.3	30.7 ± 0.4	55.0 ± 2.0	1.3 ± 0.1	160.0
CSL$^+$	72	52.8 ± 0.3	29.1 ± 0.6	59.5 ± 0.9	4.8 ± 0.2	160.0

CSL – Corn steep liquor 10% (v/v).
CSL $^+$ – Corn steep liquor 10% (v/v) supplemented with $FeSO_4$ 2.0 mM, $MgSO_4$ 0.8 mM and $MnSO_4$ 0.2 mM .

These biosurfactants were characterized as a mixture of C_{13}, C_{14} and C_{15}-surfactin.

The applicability of the biosurfactants produced by *P. aeruginosa* #112 and *B. subtilis* #573 in oil recovery was evaluated and compared with the chemical surfactants Enordet and Petrostep [2,3].

Table 3. Percentages of oil recovered using the biosurfactants produced by *P. aeruginosa* #112 and *B. subtilis* #573, and the chemical surfactants Enordet and Petrostep, at concentration 5 mg/mL.

	Oil recovered (%)
Cell-free supernatant	
***P. aeruginosa* #112**	64.2 ± 3.5
(Bio)surfactant	
***P. aeruginosa* #112**	55.0 ± 3.4
***B. subtilis* #573**	25.1 ± 1.7
Enordet	54.4 ± 5.9
Petrostep	30.5 ± 2.5
Control	0.0 ± 0.0

The best results were obtained using the biosurfactants produced by *P. aeruginosa* #112 without purification (cell-free supernatant), followed by the biosurfactant produced by *P. aeruginosa* #112 (purified) and the chemical surfactant Enordet.

CONCLUSIONS

- A medium containing CSL (10% (v/v)) and molasses (10% (w/v)) proved to be the best for biosurfactant production (3.2 g/L) by *P. aeruginosa* #112.
- A medium consisting of CSL (10% (v/v)) supplemented with $FeSO_4$ 2.0 mM, $MgSO_4$ 0.8 mM and $MnSO_4$ 0.2 mM was the best for biosurfactant production (4.8 g/L,) by *B. subtilis* #573.
- The biosurfactant produced by *P. aeruginosa* #112 was characterized as a mixture of eight different rhamnolipid congeners.
- The biosurfactant produced by *B. subtilis* #573 was a mixture of C_{13}-, C_{14}- and C_{15}-surfactin.
- Rhamnolipid mixtures were more efficient in oil recovery assays than the chemical surfactants.

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ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the projects BIOCLEAN – Desenvolvimento de produtos contendo surfactantes microbianos para limpeza e desinfeção de superfícies industriais e domésticas. Ref.: QREN-n.º 2013/030215, and NCMICROBIOS - Desenvolvimento de bioprocessos usando microrganismos não convencionais para a produção de biosurfactantes - Convénio FCT-CNPq Nº 17/2013 – Ref.: Projecto nº 6818.