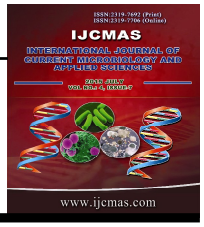
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 550-562**
http://www.ijcmas.com



Original Research Article

Bioremediation of Petroleum Derivative Using Biosurfactant Produced by *Serratia marcescens* UCP/WFCC 1549 in Low-Cost Medium

Dayana Montero-Rodríguez[1,2], Rosileide F. S. Andrade[1,2], Dafne Luana Ramos Ribeiro[2,3], Daylin Rubio-Ribeaux[1,2], Roberto A. Lima[1,2], Hélvia W. C. Araújo[4] and Galba M. Campos-Takaki[2]*

[1]Center of Biological Sciences, Federal University of Pernambuco, 50670-420, Recife, Pernambuco, Brazil
[2]Chemical Engineering Department, Federal University of Pernambuco, 50670-901, Recife, Pernambuco, Brazil.
[3]Chemistry Department, State University of Paraíba, 58429-500, Campina Grande, Paraíba, Brazil
[4]Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, 50050-590, Recife, Pernambuco, Brazil
**Corresponding author*

Keywords

Biosurfactant; bioremediation; petroleum derivatives; agro-industrial wastes; *Serratia marcescens*

A B S T R A C T

Microbial biosurfactants are found to have widely applications in environmental protection, which includes enhancing of oil recovery, oil spills control, biodegradation and detoxification of oil-contaminated industrial effluents and soils. They can be obtained with the use of agro-industrial wastes as substrates, which helps reduce overall production costs. This work was aimed for biosurfactant production by *Serratia marcescens* UCP/WFCC 1549 through bioconversion of agro-industrial residues (cassava wastewater and corn waste oil) using a 2^2 factorial design. The best production was obtained in medium containing 6% cassava wastewater and 7.5% corn waste oil, with reduction of surface tension of water to 27.8 mN/m. The crude biosurfactant showed ability to emulsify petroleum derivatives (EI_{24} > 60% of diesel, engine oil and burned engine oil), as well as be stable in a wide range of pH, temperature and salinity. Also, the crude biosurfactant exhibited excellent properties to dispersing engine oil in water (78%) and to removing burned engine oil in beach sand and mangrove sediment (88.27% and 73.70%, respectively). These results demonstrated the high potential of biosurfactant produced by *S. marcescens* as sustainable for application in bioremediation processes of hydrophobic pollutants derivated of petroleum.

Introduction

Petroleum is one of the most important energy resources around the world and its use as fuel has contributed to intensive economic development (Zhang et al., 2011;

Silva et al., 2014). However, petrochemical industry produces considerable amounts of hazardous wastes that contaminate waters and soils as a consequence of leaks and spills from petroleum refinery process, oil transportation and storage tanks (Aparna et al., 2011; Souza et al., 2014). Therefore, the environmental contamination by petroleum derivatives has been increasing over the last years, due to its use in several industrial segments (Janbandhu and Fulekar, 2011; Silva et al., 2014).

Hydrocarbons are described as extremely pollutant, toxic, with carcinogenic and mutagenic potential for humans (Janbandhu and Fulekar, 2011; Shahaby et al., 2015). The concern with these compounds increases due to difficulties in removing them from the environment (Ławniczak et al., 2013). The remediation of contaminated sites can be achieved by physicochemical or biological methods (Mulligan, 2009). Conventional physicochemical methods can rapidly remove the majority of spilled pollutants but cannot remove them completely (Noparat et al., 2014). Thus, with the advance of sustainable technologies, the search for natural method for removal and/or degradation of soil and water contaminated has increased (Aparna et al., 2011).

Biological treatment, or bioremediation, is a desirable alternative due to its cost-effectiveness and safety (Mulligan, 2009; Zheng et al., 2012; Montagnolli et al., 2015). Low solubility and high hydrophobicity of hydrocarbons make them highly unavailable to microorganisms. Several microorganisms produce biosurfactants in order to degrade hydrocarbons and use them as carbon source (Pirôllo et al., 2008; Pacwa-Płociniczak et al., 2011).

Biosurfactants are surface-active compounds produced by microorganisms, mainly aerobic ones, such as bacteria, yeasts and filamentous fungi (Muthusamy et al., 2008; Kapadia et al., 2013). There are many types of biosurfactants, based on their molecular weight and chemical structures (Banat et al., 2010). These microbial products have received considerable attention in the field of environmental remediation because they influence such processes due to their efficacy as dispersion and remediation agents. Also, their environmentally friendly characteristics, such as low toxicity and high biodegradability make them suitable for application in environments (Pacwa-Płociniczak et al., 2011; Ławniczak et al., 2013).

Despite these important advantages exhibited by biosurfactants, they have not yet been employed extensively in the industry due to their relatively high production costs (Luna et al., 2013; Banat et al., 2014). Currently, their prices range between 2 and 3 USD/kg and are 20-30% more expensive than their synthetic equivalents (Sarubbo et al., 2014). The reduction of production costs of biosurfactants requires enhancement of biosynthesis efficiency and the selection of inexpensive medium components since they constitute 50% of the total production costs. One possible strategy for reducing these costs is the utilization of alternative substrates, such as waste or by-products from the agro-industry (Makkar et al., 2011; Banat et al., 2014).

In addition, modern society produces high quantity of waste materials through activity related to industries, forestry, agriculture and municipalities (Montoneri et al., 2009). It was reported that millions of tons of hazardous and non-hazardous wastes are generated each year throughout the world and therefore there is a global concern for its management and utilization (Makkar et al., 2011). One of the possibilities explored

extensively is the use of organic matter rich but cheap agro-based raw materials or industrial wastes as substrates for microbial production. This approach will help to achieve double benefits of reducing pollution while producing useful products (Jain et al., 2013). A variety of these cheap raw materials have been employed for biosurfactant production including vegetable oils, waste frying oils, distillery and dairy wastes, cassava wastewater and corn steep liquor (Makkar et al., 2011; Andrade Silva et al., 2014; Rocha e Silva et al., 2014).

Thus, the aim of this work was to produce biosurfactants by the bacterium *Serratia marcescens* UCP/WFCC 1549 cultivated in low-cost medium in order to reduce the cost of process and evaluate its application in the removal of petroleum derivatives.

Materials and Methods

Microorganism

The bacterium *Serratia marcescens* UCP/WFCC 1549 was kindly supplied from the Culture Collection of the Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, Recife, state of Pernambuco, Brazil. The microorganism was maintained in Luria Bertani (LB) solid medium (trytone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and agar 15 g/L) at 5°C. For pre-culture, the strain from 24 h -culture on LB medium was transferred to 50 mL of LB broth and maintained under orbital shaker at 150 rpm during 18 h at 28ºC, to obtain the seed culture.

Agro-industrial wastes

The production medium was composed by cassava wastewater (CW) obtained from an industry food at municipal district of Carnaíba, state of Pernambuco, Brazil and corn waste oil (CWO), kindly supplied from a local restaurants in the city of Recife, state of Pernambuco, Brazil. The substrates were stored according to the suppliers' recommendations and used without any further processing.

Culture conditions and biosurfactant production

The experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of production medium, with varying concentrations of CW and CWO, in agreement with the experimental factorial design. After to adjust pH of the media to 7.0 and sterilization by autoclaving, each flask was inoculated with 1% (v/v) of the seed culture (0.8 optical density at 600 nm, corresponding to 10^7 cells/mL). Cultivations were carried out on a rotatory shaker at 150 rpm, for 72 h at 28°C.

A 2^2 full factorial design (FFD) was carried out to analyze the effects and interactions of CW and CWO on the surface tension as response variable, using a set of eight experiments, with four replicates at the central points (Table 1). The effects and significance of the variables were graphically illustrated using a Pareto chart. The data obtained from the experiments were subjected to statistical analysis by STATISTICA software version 7.0 (StatSoft Inc., USA) and the significance of the results was tested at $p < 0.05$ level.

Determination of surface tension

The surface tension was determined on metabolic cell-free liquid obtained by centrifugation (10,000× g for 15 min) and subsequent filtration of cultures, using a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) by the Du Nouy

ring method at room temperature (± 28° C). Measurements of surface tension from distilled water and from the conventional medium were used as control (Kuyukina et al., 2001).

Emulsification index

The emulsification index was analysed according to Cooper and Goldenberg (1987) for the better condition of the FFD, determined by measuring of surface tension. The test was done using 2 mL of the metabolic cell-free liquid and 2 mL of petroleum derivatives (diesel, kerosene, engine oil and burned engine oil) in a graduated screw cap test tube and vortexed at high speed for 2 min. After 24 h, the emulsification index (EI_{24}) was calculated by dividing the measured height of the emulsion layer by the mixture's total height and multiplying by 100 to expressing in percentage.

Stability of the biosurfactant

Stability studies were undertaken using the cell-free broth obtained after centrifugation of the cultures at 10,000× g for 15 min. Twenty millilitres of this broth were maintained at a constant temperature (0, 5, 70, 100 and 120 °C) for 1 h, and cooled to room temperature, after which the surface tension was measured. The effect of pH on surface tension was evaluated after adjustment of the broth pH to 2, 4, 6, 8, 10, 12 and 14 with 6.0 M NaOH or HCl. The effect of NaCl concentrations (2–12%, w/v) on the activity of the biosurfactant were also determined. The tests were performed in triplicate (Luna et al., 2011, 2013).

Oil displacement test

The oil displacement test was carried out by slowly dropping 1 mL of burned engine oil onto the surface of 30 mL of distillated water in a Petri dish (9 cm in diameter). This was followed by the addition of 500 μL of the cell-free metabolic liquid (crude biosurfactant) on the center of the oil layer. The mean diameter of the clear zones of triplicates experiments was measured and calculated as the rate of the Petri dish diameter (Ali Diab and El Din, 2013; Andrade Silva et al., 2014). The chemical surfactant Triton X-100 was used as control.

Bioremediation test of beach sand and mangrove sediment

Testing the suitability of biosurfactant for bioremediation process was conducted by using 60 g of beach sand or mangrove sediment impregnated with 5 mL of burned engine oil. Biosurfactant produced by *S. marcescens* cultivated in the better condition of factorial design, was used in the removal tests. Fractions of 20 g of the contaminated sand or mangrove sediment were transferred to 250 mL Erlenmeyer flasks, which were submitted to the following treatments: addition of 40 mL of the cell-free metabolic liquid and addition of 40 mL distilled water (control). The tests were carried out in triplicate and the samples were incubated on a rotary shaker (150 rpm) for 48 h at 28 °C. Then, they were centrifuged at 5000 g for 10 min for separation of the wash solution and the sand or mangrove sediment. The amount of oil residing after the impact of biosurfactant was gravimetrically determined using hexane (Luna et al., 2011).

Results and Discussion

Production of biosurfactant by *Serratia marcescens*

One of main factors governing the success of biosurfactants production is the development of an economical process that

uses low-cost materials (Rufino et al., 2014). Cassava wastewater and vegetable waste oil are agro-industrial wastes which disposal causes environmental problems; however, their high content of nutrients make them attractive substrates for several biotechnological processes (Makkar et al., 2011; Andrade Silva et al., 2014; Berger et al., 2014). Then, this paper describes the use of two residues - cassava wastewater (CW) and corn waste oil (CWO)- as low-cost medium components for biosurfactant production by *S. marcescens* UCP/WFCC 1549.

The microorganism was cultivated during 72 h, in different concentrations of CW and CWO, according to a 2^2 full factorial design (FFD). The results shown in Table 2 demonstrated that *S. marcescens* had the ability to reduce the surface tension in presence of these agro-industrial wastes, with the higher reduction in condition 4, corresponding to medium composed by 6% CW and 7.5% CWO. These results confirm those obtained previously by Montero-Rodríguez et al. (2014), with the same residues, ratifying the suitability of both wastes as alternative substrates for biosurfactant production (Alves et al., 2014).

The estimated effects of CW and CWO on surface tension, as well as the interaction between them, are shown in Pareto chart illustrated in Figure 1. As can be seen, the interaction of both substrates had a significant influence on reducing the surface tension in the culture medium. In addition, both substrates by separate were statistically significant for biosurfactant production by *S. marcescens*. These results are in accordance with the literature that affirms that physiological biosurfactant production is associated with the assimilatory mechanism in response to exposure to hydrophobic substrates, isolated or combined with a soluble substrate (Rufino et al., 2014).

Emulsifier property

The emulsifying property determines the strength of biosurfactant in retaining the emulsion of hydrocarbons or oils in water (Cooper and Goldenberg, 1987). The emulsification index (EI_{24}) was determined to biosurfactant produced by *S. marcescens* in condition 4 of the 2^2 FFD, using various hydrophobic compounds derivatives from petroleum (Figure 2). The results showed that stable emulsions were formed using diesel, engine oil and burned engine oil), confirmed by good emulsification indices up to 60% after 24 h. The maximum EI_{24} was observed with burned engine oil (72.7%). Previously, few studies had reported biosurfactant produced by *S. marcescens* strains, with good properties emulsifying kerosene as hydrocarbon (Roldan-Carrillo et al., 2011; Ibrahim et al., 2013), but this is the first study showing the ability of emulsify efficiently burned engine oil. One of the most important characteristic of hydrocarbon degrading bacteria is the ability to emulsify hydrocarbons in solution by producing surface active agents that cause dispersion of hydrocarbons in water emulsion, microdroplets or micelles which are subsequently transported into the cell (Hommel, 1990; Adebusoye et al., 2008).

Stability studies

The diverse application of biosurfactants in different fields depends on its stability at different temperatures, pH and salinity (Jain et al., 2013). For example, many hydrocarbon-contaminated environments are characterized by extreme environmental conditions such as very low or elevated temperatures, highly acidic or alkaline pH, high saline concentrations and or high pressures (Okoro et al., 2012).

Table.1 Design matrix for the factorial experiments used to evaluate the influence of two factors (cassava wastewater (CW) and corn waste oil (CWO)) on biosurfactant production by *S. marcescens* UCP/WFCC 1549, with experimental conditions set at the mean of two extreme levels (−1 and +1) and a central point (0)

Independent Variables	Factor levels		
	-1	0	+1
Cassava wastewater (CW) % (v/v)	3.0	4.5	6.0
Corn waste oil (CWO) % (v/v)	6.5	7.0	7.5

Table.2 Surface tension values obtained in the 2^2 full factorial design used for biosurfactant production by *S. marcescens* UCP/WFCC 1549 at 28°C and 150 rpm during 72 hours

Conditions	Cassava wastewater	Corn waste oil	Surface tension (mN/m)
1	-1	-1	30.5
2	+1	-1	31.6
3	-1	+1	31.5
4	+1	+1	27.8
5	0	0	32.8
6	0	0	32.2
7	0	0	32.7
8	0	0	32.5

Table.3 Removal of burned engine oil adsorbed in beach sand and mangrove sediment by the cell-free culture medium containing the crude biosurfactant produced by *S. marcescens* UCP/WFCC 1549 and by distilled water (control)

Treatments	Removal (%)	
	Beach sand	Mangrove sediment
Distilled water (control)	51.80	65.47
Cell-free culture medium (crude biosurfactant)	88.27	73.70

Figure.1 Pareto chart of standardized effects of (1) cassava wastewater (CW) and (2) corn waste oil (CWO) on the surface tension of culture media by *S. marcescens* UCP/WFCC 1549. The point at which the effect estimates were statistically significant (p = 0.05) is indicated by the broken vertical line

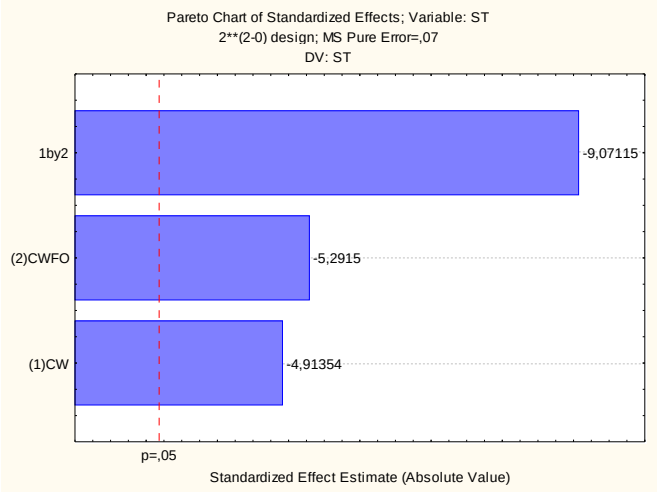


Figure.2 Emulsification index of the biosurfactant produced by *S. marcescens* UCP/WFCC 1549 in medium containing 6% cassava wastewater and 7.5% corn waste oil

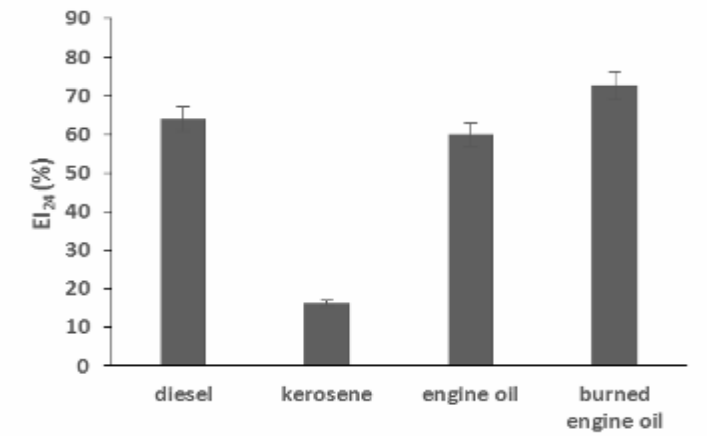
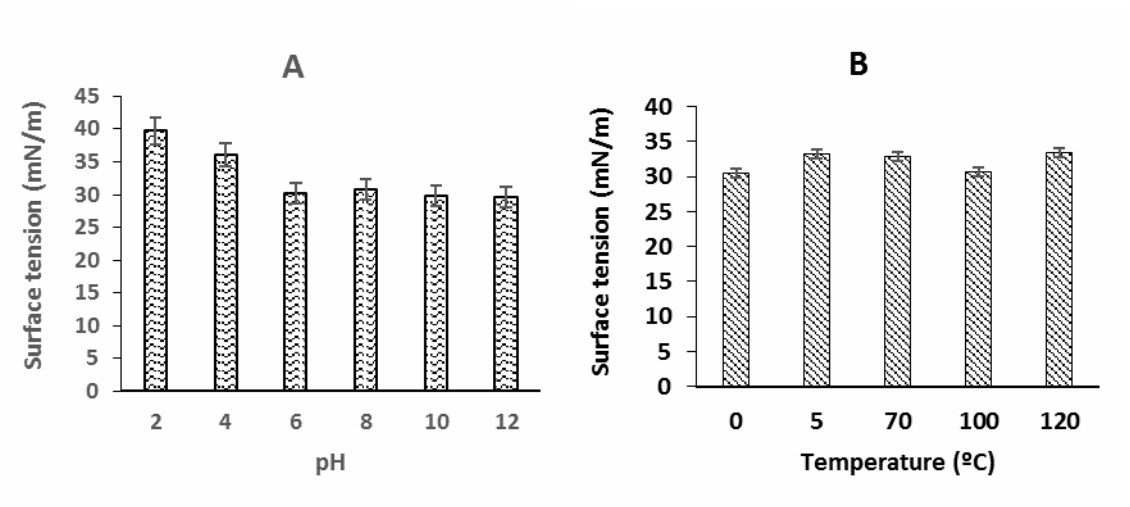


Figure.3 Stability of surface tension of biosurfactant produced by *S. marcescens* UCP/WFCC 1549 using cassava wastewater and corn waste oil. Influence of pH (**A**), temperature (**B**), and sodium chloride concentrations (**C**) on surface tension stability



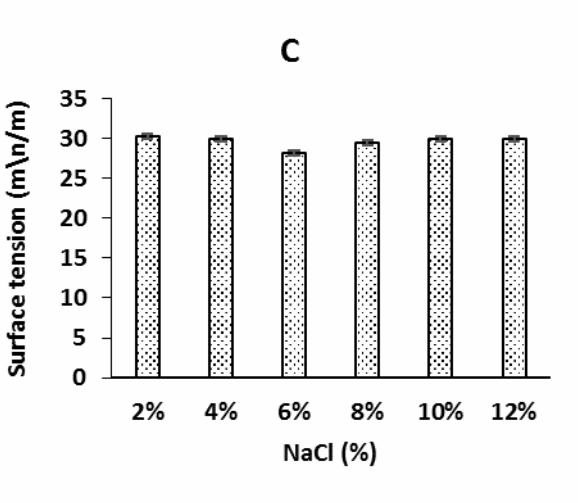


Figure.4 Oil displacement activity of crude biosurfactant produced by *S. marcescens* UCP/WFCC 1549. Engine oil droplet without application of surfactant (A), after dispersion with Triton X-100 (B) and after dispersion with crude biosurfactant produced by *S. marcescens*

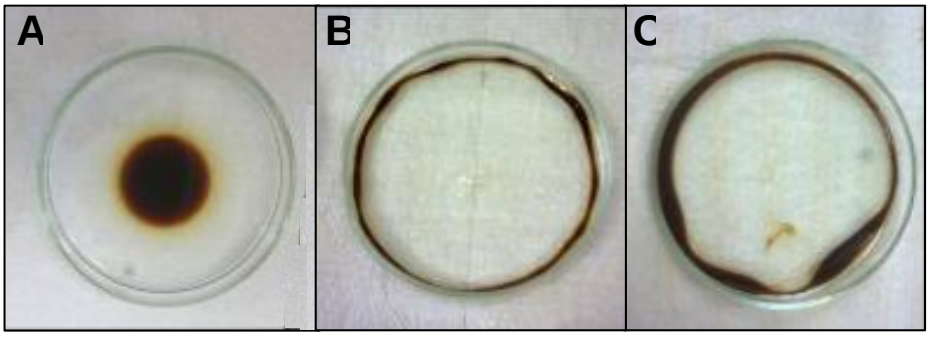


Figure.5 Bioremediation test of beach sand and mangrove sediment using crude biosurfactant produced by *S. marcescens* UCP/WFCC 1549. (A) Beach sand adsorbed with burned engine oil without treatment (control). (B) Beach sand with remaining burned engine oil after treatment with distilled water. (C) Beach sand with remaining burned engine oil after treatment with the crude biosurfactant. (D) Mangrove sediment adsorbed with burned engine oil without treatment (control). (E) Mangrove sediment with remaining burned engine oil after treatment with distilled water. (F) Mangrove sediment with remaining burned engine oil after treatment with the crude biosurfactant

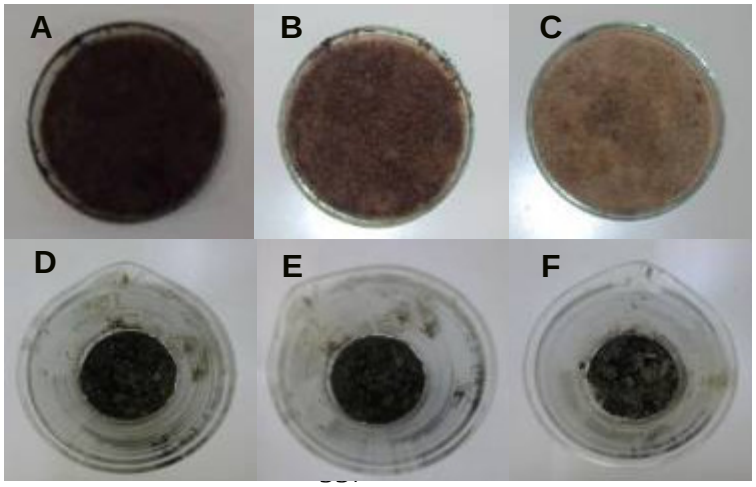


Figure 3 shows the effects of temperature, pH and salinity concentration in stability of surface tension of the biosurfactant produced by *S. marcescens*. The results showed that crude biosurfactant was stable to the neutral to basic pH range, with an increase in surface tension in acid pH (Figure 3A). In case of temperature, the surface tensions remained practically uniform at different values (0-120ºC) (Figure 3B), indicating that variation in temperature had no appreciable effect. Similarly, the addition up to 12% (w/v) sodium chloride to the cell-free liquid showed no noticeable effect in the surface tension. Biosurfactant produced by other *S. marcescens* strains have indicated stability in a wide range of pH, temperature and salinity as was demonstrated in present study, make them potential candidates for microbial enhanced oil recovery or bioremediation of environment in extreme conditions (Anyanwu et al., 2011; Ibrahim et al., 2013).

Oil displacement test

The oil displacement test is an indirect measurement of surface activity of surfactant sample tested against oil; a larger diameter indicates a higher surface activity of the testing solution (Ibrahim et al., 2013). Figure 4 illustrates the dispersant activities of Triton X-100 and crude biosurfactant produced by *S. marcescens*. The dispersion rate obtained with the use of synthetic surfactant was 89%, whereas the cell-free metabolic liquid (crude biosurfactant) achieved a dispersion rate of 78%, showing its potential in the dispersion of oil spills.

Sitohy et al. (2010) reported an oil displacement rate of 57% using a biosurfactant produced by *Bacillus subtilis* whereas Rocha e Silva et al. (2014) achieved an 80% oil displacement rate using crude biosurfactant of *Pseudomonas cepacia*. The present study shows excellent property of biosurfactant produced by *S. marcescens* UCP/WFCC 1549 which was better than those showed by others *S. marcescens* strains previously studied (Roldan-Carrillo et al., 2011; Ibrahim et al., 2013).

Bioremediation test of contaminated beach sand and mangrove sediment

The release of contaminants, such as petroleum and petroleum byproducts into the environment is one of the main causes of global pollution and has become a focus of great concern both in developing countries due to the broad environmental distribution (Mulligan, 2009; Silva et al., 2014). The marine environment has suffered with constant oil spills, making oil one of the most abundant organic contaminants in the sea (Souza et al., 2014). Half of world oil production is transported by ships through the oceans, increasing hydrocarbon contamination levels in various marine ecosystems, such as beaches and mangroves due to possible accidents (Santos et al., 2011; Silva et al., 2014; Oliveira et al., 2015).

Although conventional physicochemical methods can rapidly remove the majority of spilled oil, in most cases, these methods cannot clean up completely crude oil or the removal simply transfers contaminants from one environmental medium to another. Thus, more attention is being given to biological alternatives (Malik and Tsai, 2012; Lin et al., 2014). Biosurfactants play an important role in remediation processes

due to their efficacy as dispersion and remediation agents as well as their environmentally friendly characteristics, such as low toxicity and high biodegradability (Luna et al., 2011; Silva et al., 2014).

Figure 5 and Table 3 show the results obtained for the removal of burned engine oil adsorbed in samples of beach sand and mangrove sediments by crude biosurfactant from *S. marcescens*. In comparison with distilled water (control), promising results were obtained by crude biosurfactant from *S. marcescens* with removal of 88.27% and 73.70%, respectively.

Results described in the literature show that the biosurfactants produced by strains of *Pseudomonas aeruginosa* removed 49-54% of crude oil adsorbed in sand (Bordoloi and Konwar, 2008), whereas Silva et al. (2010) demonstrated high removal rates (above 85%) of diesel oil from sand samples, but lower (less than 20%) when petroleum was tested. Nalini and Parthasarathi (2013) showed the recovery of 92% of used engine oil adsorbed in sand by biosurfactant produced by *Serratia rubidae.* The results obtained in present study demonstrated considerable potential of crude biosurfactant of *S. marcescens* UCP/WFCC 1549 for use in bioremediation processes of oil-polluted environments.

Acknowledgements

This work was financially supported by National Council for Scientific and Technological Development (CNPq) 563382/2010-4-SISBIOTA and 308245/2010-5 (G.M.C.T.), Coordination for the Improvement of Higher Level Education Personnel (CAPES), Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE). The authors are grateful to Graduate Student Agreement Program (PEC-PG) of CNPq for the Master scholarship and to NPCIAMB-Catholic University of Pernambuco, Brazil, for use of laboratories.

References

Adebusoye, S.A., O.O. Amund, M.O. Ilori, D.O. Domeih and Okpuzor, J. 2008. Growth and biosurfactant synthesis by Nigerian hydrocarbon-degrading estuarine bacteria. International Journal of Tropical Biology 56, 1603-1611.

Ali Diab, A. and El Din, S.G. 2013. Application of the biosurfactants produced by *Bacillus* spp. (SH 20 and SH 26) and *Pseudomonas aeruginosa* SH 29 isolated from the rhizosphere soil of an Egyptian salt marsh plant for the cleaning of oil-contaminated vessels and enhancing the biodegradation of oily sludge. Afr. J. Environ. Sci. Technol. 7, 671–679.

Alves, T.S., J.P. Salgado, R.F.S. Andrade, D. Montero-Rodríguez, W.B. Ferreira, M.M. Almeida, G.M. Campos Takaki and Araújo, H.W.C. 2014. Production and Evaluation of Biosurfactant by *Serratia marcescens* UCP 1549 Using Industrial Wastes. British Biotechnology Journal, 4(6):708-719.

Andrade Silva, N.R., M.A. Luna, A.L. Santiago, L.O. Franco, G.K. Silva, P.M. de Souza, K. Okada, C.D. Albuquerque, C.A. da Silva and Campos-Takaki, G.M. 2014. Biosurfactant-and-Bioemulsifier Produced by a Promising *Cunninghamella echinulata* Isolated from Caatinga Soil in the Northeast of Brazil. Int. J. Mol. Sci. 15, 15377-15395.

Anyanwu, C.U., S.K.C. Obi and Okolo, B.N. 2011. Lipopeptide biosurfactant

production by *Serratia marcescens* NSK-1 Strain isolated from petroleum contaminated soil. J. Appl. Sci. Res. 7, 79-87.

Aparna, A., G. Srinikethan and Hedge, S. Effect of addition of biosurfactant produced by *Pseudomonas* ssp. on biodegradation of crude oil. In: International Proceedings of Chemical, Biological & Environmental Engineering. Proceedings of the 2nd International Proceedings of Chemical, Singapore, Pp. 71–75.

Banat, I.M., A. Franzetti, I. Gandolfi, G. Bestetti, M.G. Martinotti, L. Fracchia, T.J.P. Smyth and Marchant, R. 2010. Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol. 87, 427-444.

Banat, I.M., S.K. Satpute, S.S. Cameotra, R. Patil and Nyayanit, N.V. 2014. Cost effective technologies and renewable substrates for biosurfactants' production. Frontiers in Microbiology 5:697.

Berger, L.R.R., T.C.M. Stamford, T.M. Stamford-Arnaud, L. de Oliveira Franco, A.E. do Nascimento, H.M.M. Cavalcante, R.O. Macedo and de Campos-Takaki, G.M. 2014. Effect of Corn Steep Liquor (CSL) and Cassava Wastewater (CW) on Chitin and Chitosan Production by *Cunninghamella elegans* and Their Physicochemical Characteristics and Cytotoxicity. Molecules 19, 2771-2792.

Bordoloi, N.K. and Konwar, B.K. 2008. Microbial surfactant-enhanced mineral oil recovery under laboratory conditions. Colloids and Surfaces B 63, 73–82.

Cooper, D.G. and Goldenberg, B.G. 1987. Surface-active agents from two *Bacillus* species. Appl. Environ. Microbiol. 53, 224–229.

Hommel, R.K. 1990. Formation and physiological role of biosurfactants produced by hydrocarbon utilizing microorganisms. Biodegradation 1, 107-119.

Ibrahim, M.L., U.J.J. Ijah, S.B. Manga, L.S. Bilbis and Umar, S. 2013. Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. International Biodeterioration & Biodegradation 81, 28-34.

Jain, R.M., K., Mody, N. Joshi, A. Mishra and Jha, B. 2013. Effect of unconventional carbon sources on biosurfactant production and its application in bioremediation. International Journal of Biological Macromolecules 62, 52-58.

Janbandhu, A. and Fulekar, M.H. 2011. Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. J. Hazard. Mater. 187, 333-340.

Kapadia, S.G. and Yagnik, B.N. 2013. Current trend and potential for microbial biosurfactants. Asian J. Exp. Biol. Sci. 4, 1–8.

Kuyukina, M.S., I.B. Ivshina, J.C. Philp, N. Christofi, S.A. Dunbar and Ritchkova, M.I. 2001. Recovery of *Rhodococcus* biosurfactants using methyl tertiary-butyl ether extraction. J Microbiol Methods. 46 (2):149-156.

Ławniczak, Ł., R. Marecik and Chrzanowsk, L. 2013. Contributions of biosurfactants to natural or induced bioremediation. Appl. Microbiol. Biotechnol. 97, 2327-2339.

Lin, M., L. Yuhua, C. Weiwei, W. Hui and Xiaoke, H. 2014. Use of bacteria-immobilized cotton fibers to absorb and degrade crude oil. Int. Biodeterior. Biodegrad. 88, 8–12.

Luna, J.M., R.D. Rufino, C.D. Albuquerque, L.A. Sarubbo and Campos-Takaki, G.M. 2011. Economic optimized medium for tensio-active agent production by *Candida sphaerica* UCP0995 and application in the removal of hydrophobic contaminant from sand. Int. J. Mol. Sci. 12, 2463-2476.

Luna, J.M, R.D. Rufino, L.A. Sarubbo and Campos-Takaki, G.M. 2013. Characterization, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida sphaerica* UCP0995 for application in the petroleum industry. Colloids and Surfaces. B, Biointerfaces, 102, 202-209.

Makkar, R.S., S.S. Cameotra and Banat, I.M. 2011. Advances in utilization of renewable substrates for biosurfactant production. AMB Express 1, 1-19.

Malik, Z.A. and Ahmed, S. 2012. Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium. Afr. J. Biotechnol. 11, 650–658.

Montagnolli, R.N., P.R.M. Lopes and Bidoia, E.D. 2015. Assessing *Bacillus subtilis* biosurfactant effects on the biodegradation of petroleum products. Environmental monitoring and assessment 187, 1-17.

Montero-Rodríguez, D., R.F.S. Andrade, D.L.R. Ribeiro, R.A. Lima, H.W.C. Araujo and Campos-Takaki, G.M. 2014. Ability of *Serratia marcescens* UCP/WFCC 1549 for biosurfactant production using industrial wastes and fuels biodegradation In: Industrial, medical and environmental applications of microorganisms: Current status and trends. A. Méndez-Vilas (Ed.). Madrid, Pp. 211-216.

Montoneri, E.; P. Savarino, S. Bottigliengo, V. Boffa, A.B. Prevot, D. Fabbri and Pramauro, E. 2009. Biomass wastes as renewable source of energy and chemicals for the industry with friendly environmental impact. Fresenius Environ Bull 18, 219–223.

Mulligan, C.N. 2009. Recent advances in the environmental applications of biosurfactants. Current Opinion in Colloid & Interface Science 14, 372-378.

Muthusamy, K., S. Gopalakrishnan, T.K. Ravi and Sivachidambaram, P. 2008. Biosurfactants: properties, commercial production and application. Curr. Sci. 94, 736-747.

Nalini, S. and Parthasarathi, R. 2013. Biosurfactant production by *Serratia rubidaea* SNAU02 isolated from hydrocarbon contaminated soil and its physico-chemical characterization. Bioresource technology, 147, 619-622.

Noparat, P., S. Maneerat and Saimmai, A. 2014. Application of Biosurfactant from *Sphingobacterium spiritivorum* AS43 in the Biodegradation of Used Lubricating Oil. Applied biochemistry and biotechnology 172, 3949-3963.

Okoro C., A. Agrawal and Callbeck C. 2012. Simultaneous biosurfactant production and hydrocarbon biodegradation by the resident aerobic bacterial flora of oil production skimmer pit at elevated temperature and saline conditions. Life Science Journal 9, 56-364.

Oliveira, V., N.C. Gomes, A. Almeida, A.M. Silva, H. Silva, H. and Cunha, Â. 2015. Microbe-Assisted Phytoremediation of Hydrocarbons in Estuarine Environments. Microbial ecology, 69, 1-12.

Pacwa-Płociniczak, M., G.A. Płaza, Z. Piotrowska-Seget and Cameotra, S.S. 2011. Environmental applications of

biosurfactants: recent advances. Int. J. Mol. Sci. 12, 633-654.

Pirôllo, M.P., A.P. Mariano, R.B. Lovaglio, S.G. Costa, V. Walter, R. Hausmann and Contiero, J. 2008. Biosurfactant synthesis by *Pseudomonas aeruginosa* LBI isolated from a hydrocarbon-contaminated site. J. Appl. Microbiol. 105, 1484-1490.

Rocha e Silva, N.M.P., R.D. Rufino, J.M. Luna, V.A. Santos and Sarubbo, L.A. 2014. Screening of *Pseudomonas* species for biosurfactant production using low-cost substrates. Biocatalysis and Agricultural Biotechnology 3, 132-139.

Roldan-Carrillo, T., X. Martinez-Garcia, I. Zapta-Penaso, G. Castrorena-Cortes, J. Reyes-Avila, M. Mayol-Castino and Olguin-Lora, P. 2011. Evaluation of the effect of nutrient ratios on biosurfactant production by *Serratia marcescens* using a boxbehuken design. Colloids and Surfaces B; Biointerfaces 86, 284-389.

Rufino, R.D., J.M. de Luna, G.M. de Campos Takaki and Sarubbo, L.A. 2014. Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. Electronic Journal of Biotechnology 17, 34-38.

Santos, H.F., F.L. Carmo, J.E. Paes, A.S. Rosado and Peixoto, R.S. 2011. Bioremediation of mangroves impacted by petroleum. Water, Air, & Soil Pollution, 216, 329-350.

Sarubbo, L.A., N.R. e Silva, I.N.S. Ferreira, F.R. e Silva, J.M. Luna and R.D. Rufino. 2014. Production of biosurfactants by *Pseudomonas* species cultivated in low-cost substrates. *Industrial, Medical and Environmental Applications of Microorganisms: Current Status and Trends*, 153-157.

Shahaby, A.F, A.A. Alharthi and El-Tarras, A.E. 2015. Bioremediation of Petroleum Oil by Potential Biosurfactant-Producing Bacteria using Gravimetric Assay. Int. J. Curr. Microbiol. App. Sci 4(5): 390-403.

Silva, R.D.C.F., D.G. Almeida, R.D. Rufino, J.M. Luna, V.A. Santos and Sarubbo, L.A. 2014. Applications of Biosurfactants in the Petroleum Industry and the Remediation of Oil Spills. Int. J. Mol. Sci. 15, 12523-12542.

Silva, S.N.R.L., C.B.B. Farias, R.D. Rufino, J.M. Luna and Sarubbo, L.A. 2010. Glycerol as subtrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP 0992. Colloids and Surfaces B: Biointerfaces 79, 174–183.

Sitohy, M.Z., M.M., Rashad, S.F. Sharobeem, A.E. Mahmoud, M.U. Nooman and Al Kashef, A.S. 2010. Bioconversion of soy processing waste for production of surfactants. African Journal of Microbiology Research, 4, 2811-2821.

Souza, E.C., T.C. Vessoni-Penna and Souza Oliveira, R.P. 2014. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. Int. Biodeterior. Biodegrad. 89, 88–94.

Zhang, Z., Z. Hou, C. Yang, C. Ma, F. Tao and Xu, P. 2011. Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated *Pseudomonas aeruginosa* DQ8. Bioresour. Technol. 102, 4111–4116.

Zheng, G., A. Selvam and Wong, J.W. 2012. Oil-in-water microemulsions enhance the biodegradation of DDT by *Phanerochaete chrysosporium*. Bioresource technology 126, 397-403.