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Biosurfactant Production by Bacteria Isolated from Seawater for Remediation of Environments Contaminated with oil Products

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Biosurfactants are a cleaning option for environments contaminated with oil. This compounds of microbiological origin have low toxicity. In pursuit of this biocompound, seawater samples were collected, diluted and striated in Petri dishes containing agar nutrient for bacterial growth. After bacteria isolation an screening was made to studied its potential. Subsequently, biosurfactant production by a bacterium isolated from seawater was performed by varying the carbon source (glucose, soybean oil and residual soybean oil frying) and nitrogen (sodium nitrate, potassium nitrate, urea and peptone). The identification of the isolates was made by mass spectrometry using Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF). After selection of the media that showed best results, the biosurfactant produced by Bacillus sp. was studied from the best growing conditions, varying growth time (48, 72 and 96 hours) and the stirring speed (150, 200 and 250 rpm).The parameters evaluated were the determination of surface tension in mN/m and the concentration in g/L. The results showed that the medium containing potassium nitrate and frying oil at 200 rpm for 72 hours showed the best results. A surface tension of 29 mN/m and a concentration of 3.6 g/L were observed. Tests under extreme conditions of pH, temperature and NaCl indicated the stability of the biosurfactant for use in the treatment of oil-contaminated environments. Washing experiments involving soil contaminated with motor oil demonstrated recovery rates greater than 90 %. The crude biosurfactant was capable of dispersing approximately 74 % of oil droplets in seawater, demonstrating that the biosurfactant can be used in future removal tests of oily compounds in industrial processes.

1. Introduction

The search for oil as an energy source increases with increasing world industrialization. Oil spills caused by oil exploration and transportation, however, often occur around the world. (Silva et al., 2014). Among the methods of recovery of the areas affected by the petroleum derivatives are the physicochemical techniques, which are not completely effective in the removal of oil spills (Rocha e Silva et al., 2015). The biological treatment technique that uses microorganisms to degrade hydrophobic pollutants, known as bioremediation, on the other hand, has advantages such as low cost environmental technology and can be an attractive alternative in solving problems related to hydrocarbon pollution (Silva et al., 2014).
Microbial compounds that exhibit marked surface activity are classified as biosurfactants, agents capable of reduce surface tension at the air-water interface and between immiscible liquids, or at the solid-liquid interface (Sarubbo et al., 2012). Characteristics such as excellent detergency, emulsification, foaming and dispersion, wetting and penetrating action, thickening capacity, increasing microbial growth, metal sequestration and oil recovery give great versatility to these biomolecules making them candidates to replace synthetic surfactants. Biosurfactants also have many advantages over surfactants of chemical origin, such as low toxicity, stability against a wide range of pH and high temperatures, as well as resistance to high saline concentrations (Brasileiro et al., 2015)

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Optimization of biosurfactant production is of paramount importance, considering its ecological acceptability, biodegradability and extensive applications. This would help reduce the cost of production in order to increase its economic competitiveness compared to synthetic surfactants (Sarubbo et al., 2015).
In this sense, the present study aims to characterize a biosurfactant produced by a bacteria isolated from the port region with the purpose of evaluating its stability under extreme conditions, as well as its potential of application in the remediation of oil derivatives in sea water.

2. Materials and Methods

2.1 Collection of sea water and isolation of bacteria

Sea water was collected in the port region of Recife - Pernambuco, Brazil. The sample was taken to the laboratory where it was submitted to a serial dilution. From each dilution, 1 ml was removed to be homogenized in Petri dishes containing the Nutrient Agar (AN) medium in order to facilitate the isolation of the bacteria.

2.2 Screening of biosurfactant productor bacteria

After the isolation of seawater bacteria, they were evaluated for the potential of biosurfactant production through hemolytic activity. This activity was determined on Petri dishes containing Agar Blood medium previously sterilized at 121 °C for 20 minutes and added with 5 % (v / v) defibrillated sheep blood.
After scoring, they were incubated at 28 °C for 24 hours. The presence of activity was detected from the formation of a halo around the colony, indicating the potential of biosurfactant production.

2.3 Biosurfactant production

The fermentations for production of the biosurfactant were carried out in Erlenmeyer flasks containing mineral medium and 5 % of the bacteria in Nutrient Broth (NB) maintained during orbital stirring at 200 rpm for 48 hours at 28 °C. After fermentation, the metabolic broth was centrifuged at an orbital velocity of 4000 rpm for 30 min to determinate the surface tension and yield of the biosurfactant.

2.4 Influence of carbon and nitrogen sources

The mineral medium was supplemented with glucose, soybean oil and soybean frying residual oil, separately, at 2 % concentration. The material produced was analysed for surface tension and yield of production. After selecting the carbon source, it was used with the mineral medium that was also supplemented with different sources of nitrogen (ammonium chloride, sodium nitrate, urea, peptone and potassium nitrate) and tested alone at a concentration of 0.12 %.

2.5 Influence of culture time and agitation speed on biosurfactant production

After the selection of the best culture medium, the influence of the fermentation time (48, 72 and 96 hours) and the agitation speed (150 200 and 250 rpm) on the biosurfactant production was evaluated.

2.6 Effect of environmental factors on biosurfactant activity

The effect of different temperatures (5 °C, 70 °C, 100 °C and 120 °C), concentrations of NaCl (2,0, 4,0, 6,0, 8,0 and 10,0 %) and pHs (2,0, 4,0, 6,0, 8,0, 10,0 and 12,0) at biosurfactant activity were available in cell-free broth through surface tension and emulsification determination. The methodology used was described by Santos et al. (2013) and Jadhav et al. (2013).

2.7 Surface tension and emulsification measurement

Changes in surface tension were monitored in the cell-free broth obtained by centrifuging the cultures for 30 min by the ring method using a Sigma 700 Tesiometer (KSV Instruments LTD – Finland) at room temperature.
The emulsification methodology used in this work was described by Cooper & Goldenberg (1987).

2.9 Yield measurement

An equal volume of $CHCl_3/CH_3OH$ (2:1) was added to cell-free broth, the mixture was vigorously shaken for 15 minutes and allowed to set until phase separation. The organic phase was obtained and quantified by gravimetric analysis.

2.10 Determination of oil removal activity on contaminated soil

In 250 ml Erlenmeyers flasks were added 50 grams of standard sand (NBR 7214) impregnated with 10 % of petroleum by-products (residual motor oil and non-residual motor oil). After the addition of 50 mL of cell

metabolic fluid, the dynamic shaker assay was performed at a rotation of 200 rpm for 24 hours. The supernatant was discarded on washed soil with 50 ml of hexane. This was taken to the heating plate and evaporated at 40 ºC, thus realizing a quantification of the oil removed by gravimetry.

2.11 Oil displacement test

The oil displacement test was carried out by slowly dropping 15 µm of motor oil onto the surface of 40 ml os distilled water in a Petri dish (15 cm in diameter) until covering the entire surface area of the water. This was followed by the addiction of 10 µL of the cell-free metabolic broth onto the surface of the oil layer. The mean diameter of the clear zones of triplicate experiments was measured and calculated as the rate of the Petri dish diameter.

3. Results and discussion

3.1 Screening of biosurfactant productor bacteria

Hemolytic activity, according to Carrillo et al. (1996) shows a relation with the amphipathic properties of the biosurfactants, being a predictive factor for the primary detection of the production of these biomolecules, mainly in bacteria. The strains isolated (Figure 1) were submitted to the evaluation of biosurfactant production by determination of hemolytic activity. From the colonies tested, it was possible to detect hemolytic activity in five strains, still unknown, from the formation of a halo around the colony, indicating the potential for biosurfactant production.

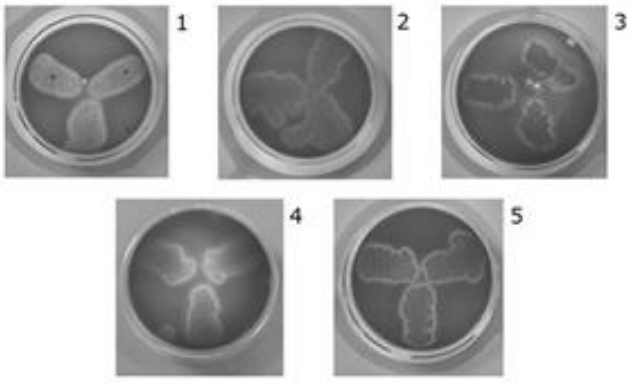

Figure 1: Indicative halos of hemolytic activities presented in 5 isolated strains.

3.2 Influence of carbon and nitrogen sources

Based on the previous results, it was possible to observe that each strain presented different values of surface tension and yield in the presence of the different carbon sources (Figures 2A and 2B) and nitrogen (Figures 3A and 3B), respectively.

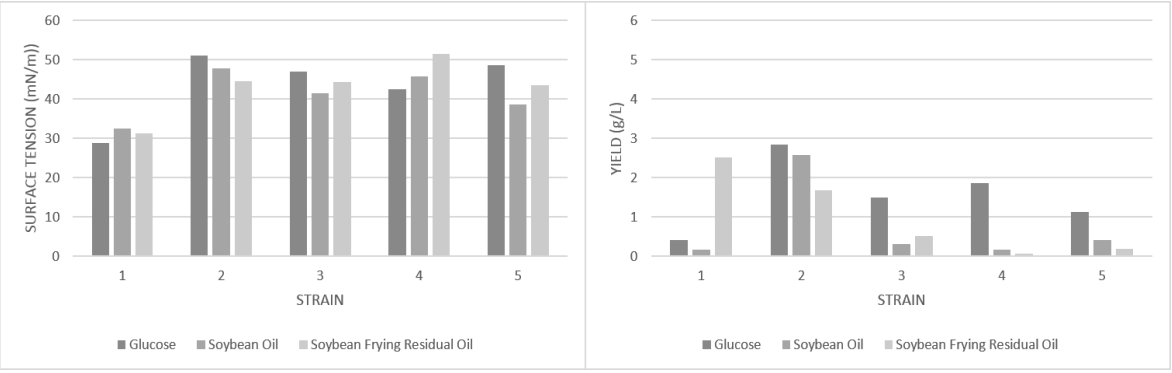

Figure 2: Evaluation of surface tension (A) and yield (B) of metabolic liquids containing biosurfactants produced by isolated microorganisms in the presence of different carbon sources.

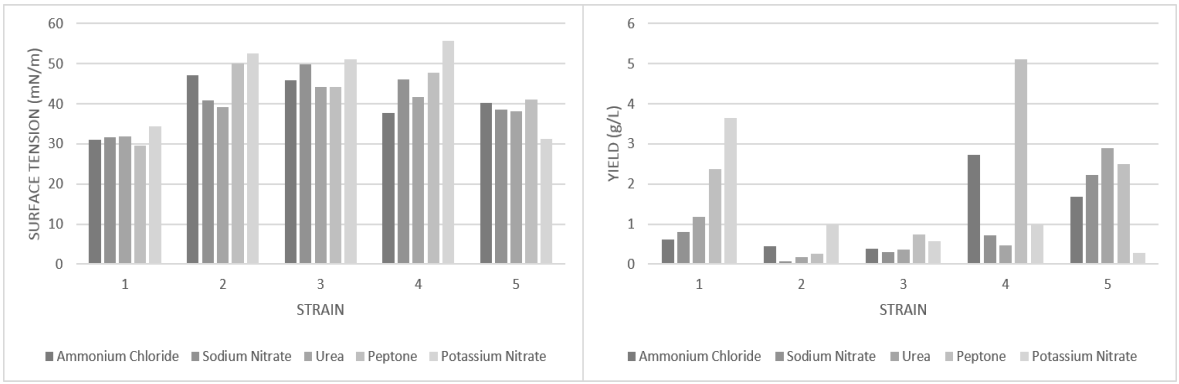

Figure 3: Evaluation of surface tension (A) and yield (B) of metabolic liquids containing biosurfactants produced by isolated microorganisms in the presence of different nitrogen sources.

Based on the results obtained, the strain 1 was selected as a biosurfactant producer in a production medium supplemented with frying residual oil and potassium nitrate. Afterwards, the selected microorganism was characterized by mass spectrometry, confirming that it is the microorganism *Bacillus sp*.

3.3 Influence of culture time and agitation speed on biosurfactant production

The results of the influence of the agitation speed and cultivation time on the surface tension of the biosurfactant produced through the strain isolated from the seawater and selected by its tensoactive potential can be observed in the Figure 4. From these results, it was possible to observe that the values of the surface tension did not present great variations, being approximately between 28 and 35 mN/m, numbers considered satisfactory according to the literature for the biocomposite in question (El-Sheshtawy et al., 2015; Mouafi et al., 2016). It may also be noted that the lower surface tensions were observed at the 72 hour culture time, which is the most considered time to be selected among the culture conditions for the production of the biomolecule.

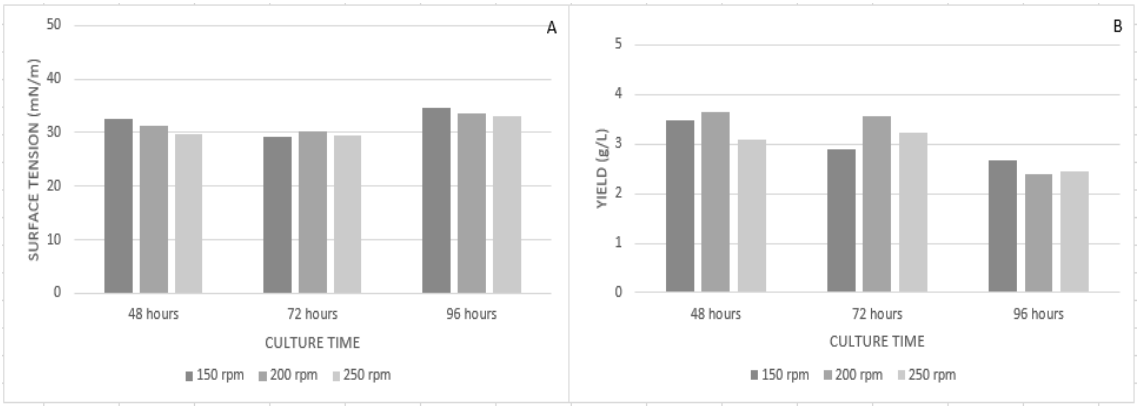

Figure 4: Influence of culture time on surface tension (A) and yield (B) of cell free metabolic fluid at different stirring rates.

3.4 Effect of environmental factors on biosurfactant activity

The microbiological surfactant showed to be very effective in its stability in all the studied conditions both in its surface tension (Figure 5) and in the emulsion (Figure 6), demonstrating a high capacity of application. This may be due to the bacteria being native to seawater.

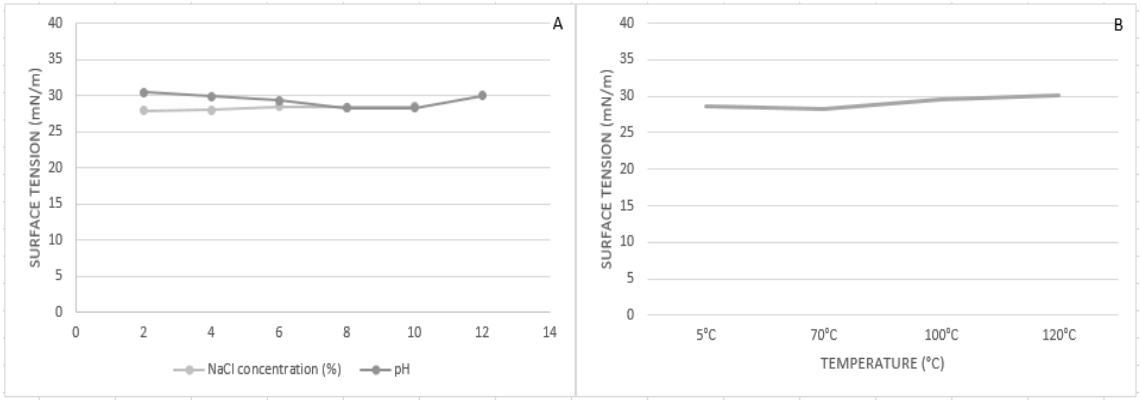

Figure 5: Variations influence of NaCl, pH (A) and temperature (B) on the surface tension of the selected biosurfactant .

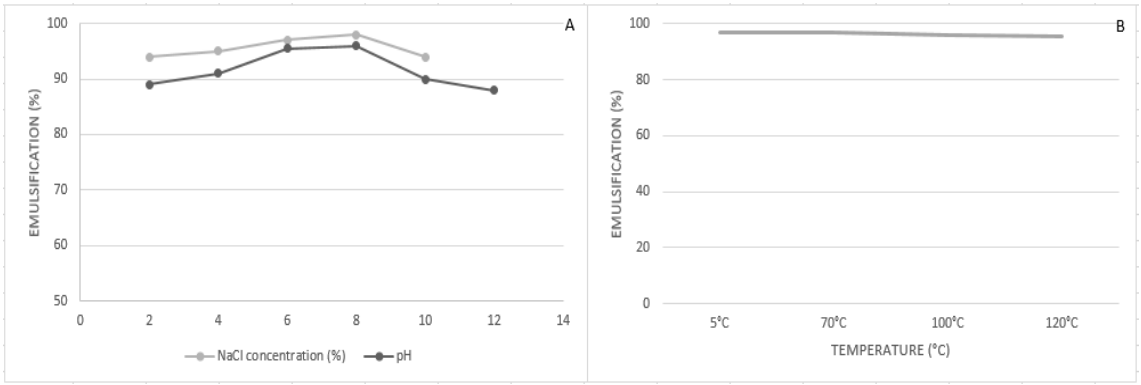

Figure 6: Variations influence of NaCl, pH (A) and temperature (B) on the emulsification of the selected biosurfactant.

3.5 Determination of oil removal activity on contaminated soil

When toxic products are introduced into soil, as in the case of petroderivatives, it can alter the physico-chemical properties of the product, causing a series of reactions of harmful effects on the ecosystem. Figure 7 shows percentage of removal in dynamic test by cell free broth with values considered quite satisfactory.

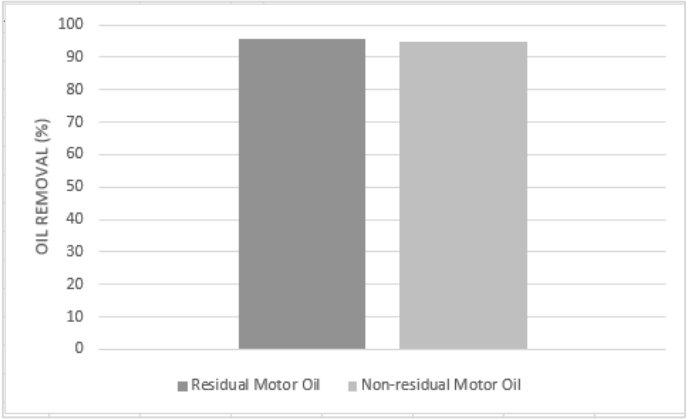

Figure 7: Percentage of oil removal by Bacillus sp. broth after 24 h.

3.6 Oil displacement test

The application potential of the biosurfactant cell-free broth for residual oil displacement in seawater was evaluated and indicate an efficiency of 74% as shown on Figure 8.

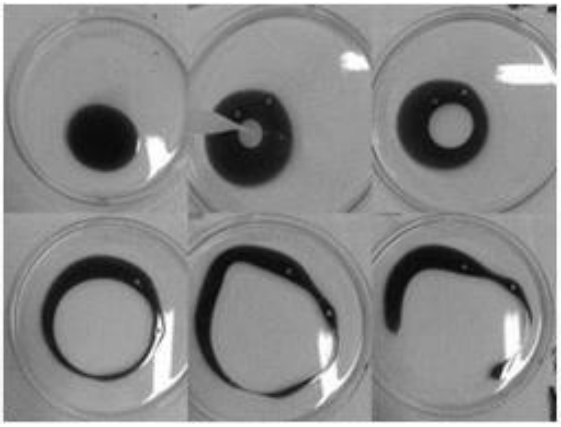

Figure 8: Determination of the activity of dispersion of motor oil by the biosurfactant produced by Bacillus sp.

4. Conclusion

The present study demonstrates the efficiency of the biosurfactant produced by Bacillus *sp*. His efficiency in the tests of influence of different variables for the biopolymer production conditions was demonstrated by his stability in the same extreme conditions of salt concentration, temperature and differentiated values of pH.

Acknowledgments

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