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# Conservation of the Biosurfactant Produced by *Pseudomonas aeruginosa* for Environmental Applications

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The biosurfactants have a molecular structure with hydrophilic and hydrophobic groups that exhibit properties such as adsorption, formation of micelles, emulsion formation, detergency and solubility, all related to the ability to reduce surface tension by these molecules.The discovery of new biosurfactants, development of new fermentation and recovery processes and the use of cheap raw materials (specifically the use of agro-industry wastes as carbon sources) will allow that more inexpensive biosurfactants can be available for remediation process. The main objective of this study was to use different conservation methods for the biosurfactant produced by *Pseudomonas aeruginosa* UCP0992. The biopolymer produced was subjected to various preservation methods: tyndalization, flowing steam plus potassium sorbate and only the sorbate. These methods served to evaluate the maintenance of biosurfactant activities against the different storage conditions. The main properties of the biopolymer as surface tension, emulsification activity and motor oil dispersion, were tested over 120 days, to ascertain the preservation method most suitable for the product. Extreme environmental conditions such as different pH, salt concentrations and temperature were also evaluated. The results showed that the biosurfactant maintained its tensoactive properties during 120 days at all conditions tested. Positive results were observed mainly for the dispersion of the engine oil by the biosurfactant stored with sorbate only, with values above 100% dispersion at the beginning of the experiment and 70% after 120 days. Given the results, it can be seen that the biosurfactant produced no change in its activities, even when submitted to different storage conditions, which makes it very promising for future use in bioremediation processes impacted by crude oil or oil environments.

## 1. Introduction

Oil refineries, as well as other large-scale industrial processes, are potential sources of environmental pollution. The report of the joint committee to review the accident at Petrobras/repar (CREA-PR) cites 33 accidents with oil spill and its derivatives in Brazil, in the period from 1975 to 2001, adding infective million liters of soil, rivers and sea (Gonzini et al., 2010; Sen, 2008; Santos et al., 2013).

The bioremediation can be defined as a process of stimulation of natural biodegradation situations for cleaning oil spills and treatment of terrestrial and aquatic environments contaminated with xenobiotics (Mukherjee et al. 2006). Accordingly, the use of surfactant compounds becomes an attractive alternative for removing hydrophobic contaminants generated by the oil industry. The surfactants are amphipathic compounds that particionam, preferably at the interface between fluid phases with different degrees of polarity and having many industrial applications. The surfactants have a molecular structure with hydrophilic and hydrophobic groups that exhibit properties such as adsorption, micelles, micro and macro formation of emulsions, foaming action, solubility and detergency, all related to the ability to reduce the surface tension by these molecules (Seydlová Svobodová , 2008; Torres et al., 2011).

A wide variety of micro organisms such as bacteria, yeast and filamentous fungi are able to produce a broad spectrum of products of products with excellent surface active properties (Silva et al., 2010). Most microbial surfactants are complex molecules, comprising different structures that include peptides, glycolipids, glycopeptides, fatty acids and phospolipids, as reviewed recently (Silva et al., 2013). In recent years, studies

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have focused on the production of biosurfactants have intensified due to the characteristics of these compounds as biodegradability, low toxicity, specificity and stability under extreme conditions of temperature, pH and salinity (Felse et al., 2006; Mukherjee et al., 2006; Rufino et al., 2008; Sarubbo et al., 2015). Nevertheless, from an economic standpoint, biosurfactants are not yet competitive with the synthetics. Biosurfactants can only replace synthetic surfactants if the cost of the raw material and the process is minimal (Rufino et al., 2013). As stated by Copper and Goldenberg (1987), to justify the replacement of synthetic surfactants by biological molecules, it is necessary to find a more economical production process. Different routes should be examined to reduce production costs, such as high yields and product accumulation, economical engineering processes, and use of cost-free or low cost feedstocks for micro organism growth and surfactant production (Santos et al., 2013). In this context, the petroleum and petrochemical industries stand out as major fields of application of biosurfactants. In order to increase the application of microbiological surfactants, methods of possible cost reductions have been sought. Currently, their prices range between 2 and 3 USD / kg and are 20-30% more expensive than their synthetic equivalents (Rufino et al., 2013). The reduction of production costs of microbiologically based surfactants requires enhancement of biosynthesis efficiency and the selection of inexpensive medium components since they constitute 50% of the total production costs (Luna et al., 2013). The Petrochemical Complex SUAPE has allowed the State of Pernambuco achieve high levels of development due to the installation of numerous economic enterprises. The installation of the Abreu e Lima refinery, however, has aroused the concern of the industries located in its surroundings, which are subject to imminent danger of spillage or leakage of these products. Therefore, it is necessary the development of technological strategies to prevent unwanted problems caused by possible environmental accidents. In this case, the development of a technology for applying the containment biosurfactant and degradation of waste petroleum is presented as a solution to prevent damage to the marine environment. This strategy, however, must ensure the capacity of detergency, emulsification, lubrication, solubilization and phase dispersion of biosurfactants stable to guarantee the conservation of marine resources.

## 2. Materials and methods

### 2.1 Microorganism

The strain of *Pseudomonas aeruginosa* UCP0992 was obtained from the culture collection of the Catholic University of Pernambuco, Brazil. The cultures were maintained on nutrient agar slants at 4 °C.

### 2.2 Growth conditions

The pre - inoculum of *Pseudomonas aeruginosa* was prepared by transferring cells grown on a slant to 50 ml of nutrient broth. The seed culture was incubated for 24 h at 37 ºC and agitated at 250 rpm. The bacterium was cultivated in a submerged culture with shaking in a New Bruswick C-24 shaker. The basal medium was composed of 2.5% ground nut oil refinery residue and 2.5% corn steep liquor dissolved in distilled water. The medium was sterilised by autoclaving at 121 ºC for 20 min. The final pH of the medium was 7.0. The inoculum (1%, v/v) was added to the cool medium. Cultivation was carried out in Erlenmeyer flasks at 37 ºC with shaking at 250 rpm for 120 h. Samples were withdrawn for analyses at regular intervals. All assays were carried out in triplicate and did not vary more than 5%.

### 2.3 Determination of emulsification index

The emulsification index was determined using the method described by Cooper and Goldenberg (1987), whereby 2 ml of a hydrocarbon (motor oil, corn oil and soybean oil) were added to 2 ml of the cell-free culture broth in a graduated screwcap test tube and vortexed at 7500rpm for 2 min. Emulsion stability was determined after 24 h and the emulsification index was calculated by dividing the height of the emulsion layer by the total height of the mixture and multiplying by 100

### 2.4 Surface tension

The surface tensions were measured by the DU NUOY ring method in the KSV Sigma 700 tensiometer (Finland). This method consists in verify the surface tension (mN/m) through the ascension of the ring until the imminence of the breaking from the surface tension

### 2.5 Stabilization of the biotensoative

The cell-free broth was submitted to conservation methods, which were tested for its surfactant properties during 0, 15, 30, 45 and 90 days. In each day, the pH was changed to 5, 7, and 9, the salt NaCl was added to 1, 3, and 5%, and the biosurfactant was submitted to heating of 30 min at 40, and 50 °C. For each analysis, the surface tension and emulsification index were evaluated.

## 3. Results and discussion

### 3.1 Stability of the biosurfactant properties

An emulsion is formed when one liquid phase is dispersed as microscopic droplets in another (continuous) liquid phase. Most microbial surfactants are substrate specific and solubilise or emulsify different hydrocarbons at different rates (Rufino et al. 2011). The poor emulsification of some hydrocarbons may be due to the inability of the biosurfactant to stabilise the microscopic droplets. Environmental factors, such as pH, salinity and temperature, also affect the activity and stability of biosurfactants and it is therefore important to study the influence of these parameters when considering specific applications for these compounds (Luna et al. 2013). In this work, the surfactant properties were evaluated on each day (0, 15, 30, 45 and 90 days) under different conditions of temperature, pH, and salinity.

The cell-free broth containing the biosurfactant produced from *Pseudomonas aeruginosa* demonstrated a surface tension of 27.46 mN/m, showing stability after the addition of potassium sorbate at 0.2% (Figure 1).Regarding the pH variation, it was observed a reduced surface tension for the cell-free broth when it was submitted to pH 5, while it was not observed a reduction in the surface tension at pH 9 (Figure 1A).

The resistance of the biosurfactant to NaCl addition (Figure 1B) and variation of temperature (Figure 1C) was also investigated. The surface tension of the cell-free broth containing the biosurfactant remained practically unchanged over salt concentrations and temperature tested.

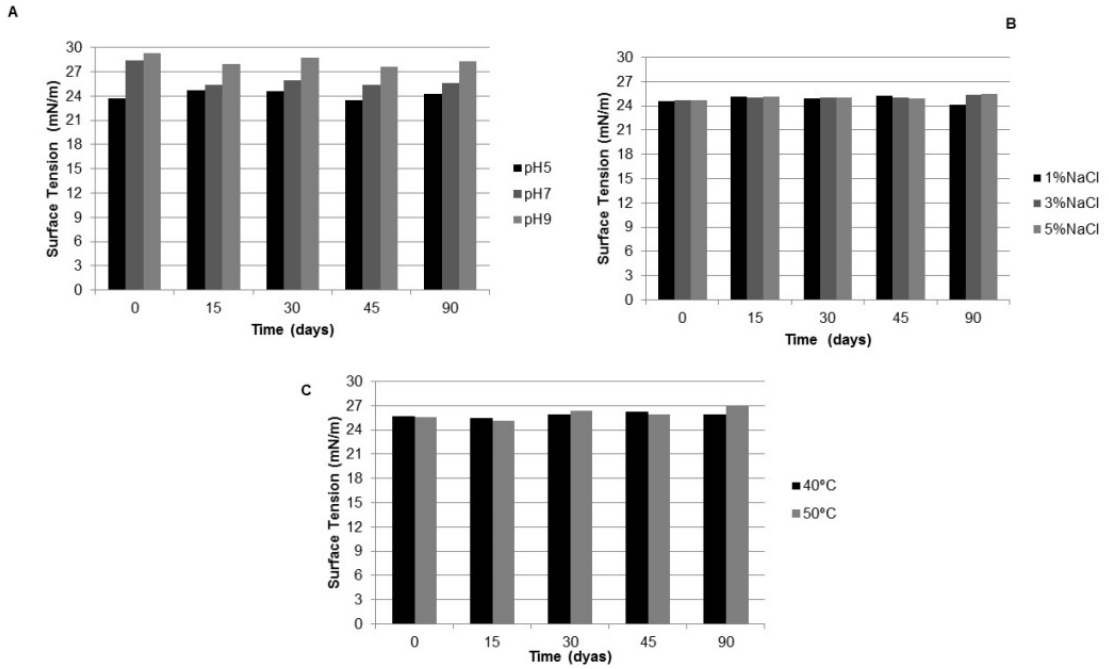


*Figure 1: Surface tensions from the biosurfactant produced by Pseudomonas aeruginosa under 0.2 % potassium sorbate during 90 days, measured in the control, pH (A), salinity (B) and heating (C) conditions*

In tests for determining the emulsification index it was observed that in all conditions tested, the biosurfactant produced emulsification values between 90% to 100% of motor oil (Figure 2). Figure 2B shows that at pH 9 (Figure 2C) the biosurfactant could emulsify well corn oil and soybean until reaching 88.23 % of the oils compared to pH 7 (Figure 2B) and pH 5 (Figure 2A).

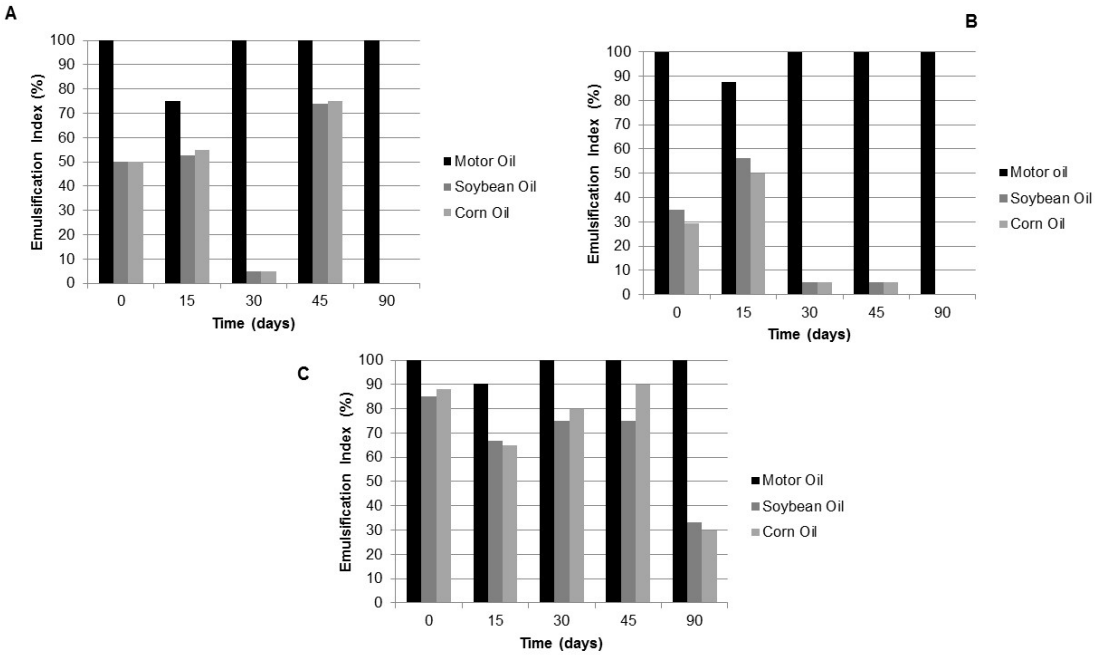


*Figure 2: Emulsification index from the biosurfactant produced by Pseudomonas aeruginosa under 0.2% potassium sorbate, during 90 days, measured in the control, pH 5 (A), pH 7 (B) and pH 9 (C) conditions in the oils: motor, soybean, and corn*

For the addition of NaCl (Figure 3), it was observed that the biosurfactant showed the best emulsification percentage of 62.5% for corn oil after addition of 1% NaCl to the cell-free broth after 15 days of the experiment (Figure 3A). Already at a concentration of 3% NaCl (Figure 3B) rates of emulsification remained constant, with a small drop on the 30 day. For the condition with 5% NaCl (Figure 6C) good emulsification indexes were observed on the 15 day.

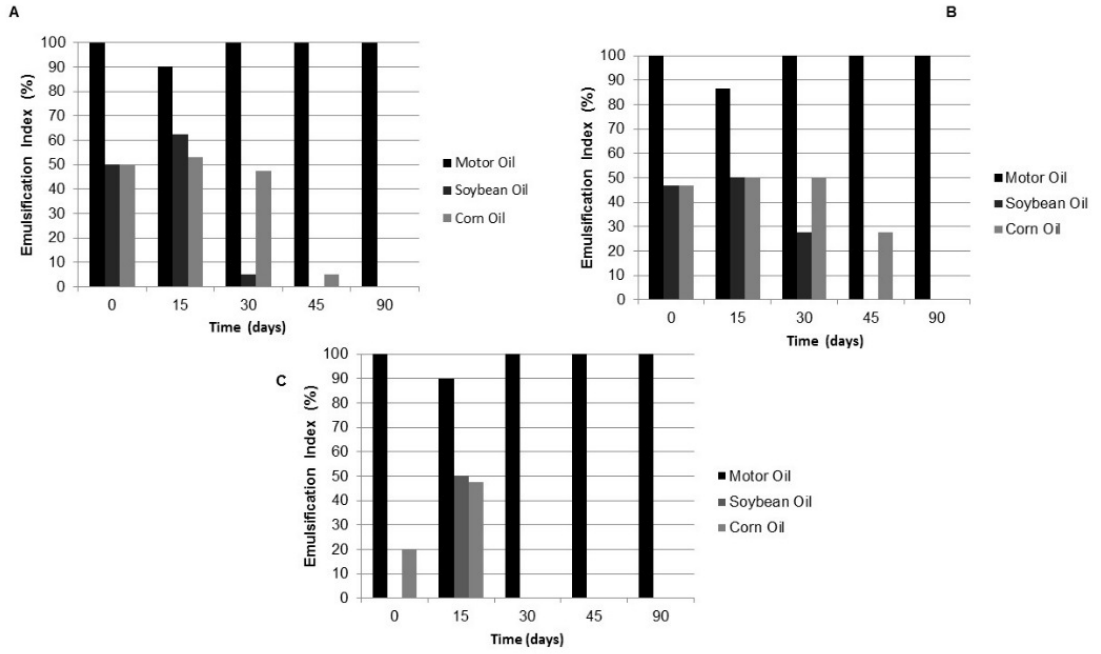


*Figure 3: Emulsification index from the biosurfactant produced by Pseudomonas aeruginosa under 0.2% potassium sorbate, during 90 days, measured in the control, 1% NaCl (A), 3%NaCl (B) and 5%NaCl (C) conditions in the oils: motor, soybean, and corn*

The stability of the biosurfactant was tested over a wide temperature range (Figure 4). Emulsification with motor oil remained stable along 90 days. It was observed that there was a gradual decrease in emulsification activity of both corn oil and soybean oil. For the temperatures of 40 ºC e 50 ºC (Figure 4A and 4B), it was observed emulsifications with values of 75 % and 70 % of corn oil and soybean, respectively at the zero time.

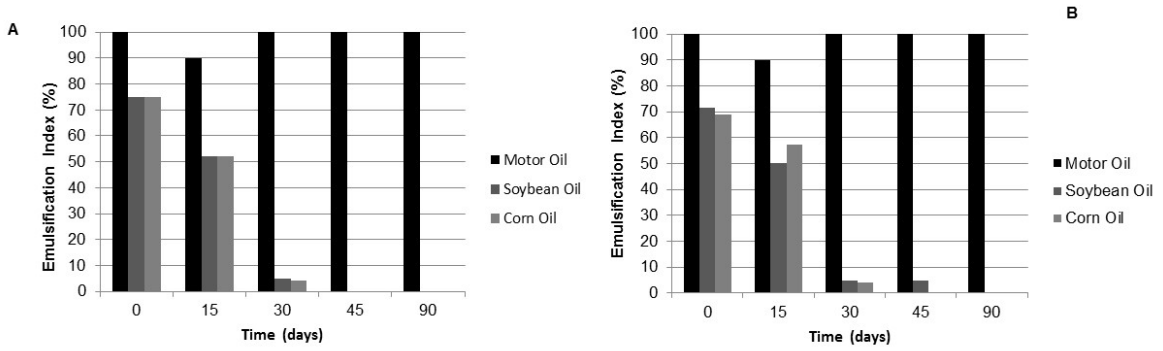


*Figure 4: Emulsification index from the biosurfactant produced by Pseudomonas aeruginosa under 0.2% potassium sorbate, during 90 days, measured in the control, 40 °C (A) and 50 °C (B) conditions in the oils: motor, soybean, and corn*

## 4. Conclusions

The biosurfactant obtained herein has potential to be used in environmental applications. Emulsification and surface tension properties of the biosurfactant show that the biomolecule is stable in environments with high pH and salinity and thus could be applied in bioremediation activities.

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