STABILITY OF A BIOSURFACTANT PRODUCED IN BIOREACTOR BY FRACTIONATED TINDALIZATION

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ABSTRACT

An environmental product to clean any area should have a low toxicity, and a high biodegrability as the microbial surfactants which came to replace their chemical counterparts. Thus, in this paper the yeast *Candida guilliermondii* was used to metabolize the industrial residues molasses, corn steep, and soybean oil, and synthesize a biosurfactant. Fermentations were carried out in a bioreactor (50 L) during 144 h, with a inoculum of 10^4 cells/mL at 28 °C, and at 200 rpm in a bioreactor. The cell-free broth was submitted to a conservation method of fractionated tindalization, and its properties were tested during 120 days. In each period of evaluation, the conditions of heating, pH, and salinity were changed to verify the surface tension, and the emulsification and dispersion indexes as parameters to evaluate the quality of the biosurfactant. The results showed the lowest tension at 26.68 mN/m, 100% of motor oil emulsification and the highest dispersion index at 97.2%. The biosurfactant produced by *Candida guilliermondii* is, therefore, a potential

biomolecule to treat contaminated areas with petroleum derivates, mainly the sea zones.

Key-words: *Candida guilliermondii*, Surface Tension, Industrial Residues.

INTRODUCTION

The oil spills accidents occur when the human being do not have the care to keep safely the petroleum outside the sea. As an example, in 2010, 800 million liters of petroleum were spoiled on the sea spreading a contamination by 1.5 km and killing many marine life. When these environmental disasters happens, it's necessary to remediate the contaminated area until 24 h after the spill, so some scientists are using chemical dispersants to control the oil slicks. These compounds are not the best solution, because the mix of petroderivates and chemical surfactants is also extremely toxic. The key, however, is to use microorganisms with biotechnological potential to degrade industries residues, and synthesize safety dispersants called biosurfactants, known as the “petroleum bioremediators” (ZHOU et al., 2013).

The biosurfactants include glycolipids, lipopeptydes, phospholipids, and other organic compounds (GAUTAM; TYAGI, 2006) which form two segments in the biomolecule: one hydrophobic and other hydrophilic. The hydrophilic moiety shows affinity for non-polar compounds, while the hydrophobic portion can be aggregated with

polar substances, so the microbial surfactant can be also called as an amphiphilic compound (MARCHANT; BANAT, 2012).

The properties of surfactants are measured mainly by the surface and interfacial tensions. The surface tension can evince the level of humectation from any liquid according with the kind of the intermolecular bonds, while the interfacial tension expresses the intensity of aggregation between hydrophilic and hydrophobic phases. Furthermore, these features can predict other properties from the biosurfactants as detergence, emulsion, dispersion, whatever (DALTIN, 2011).

The stability is another fundamental factor for the biosurfactant's production, because if a bioproduct does not maintain the initial properties for many days, it will be so expensive to synthesize it periodically. Thus, it's important to apply conditions as one pretreatment in order to preserve all features.

In this paper, the biosurfactant produced by *Candida guilliermondii* in bioreactor was submitted into a fractionated tindalization with the aim to preserve the surfactant's properties. Moreover, the conditions of heating, pH, and salinity were changed to formulate the best condition of application of the biosurfactants in sea water.

MATERIAL E METHODS

Microorganism

The yeast *Candida guilliermondii* (UCP 995) was kindly supplied by the Banco de Culturas do Núcleo de Pesquisas Ambientais from the Catholic University of Pernambuco was the biosurfactant producer. The yeast was maintained at 5 C on Yeast Mold Agar (YMA) slants containing (w/v) yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), D-glucose (1.0%), and agar (5.0%). Transfers were done to fresh agar slants each month to maintain viability (SANTOS et al., 2013).

Inoculum preparation

The *C. guilliermondii* was grown in solid medium at 27 C for 48-72 h, then, a loopful of the cells were transferred to Erlenmeyer flasks of 250 mL containing 50 mL of the Yeast Mold Broth (YMB) and incubated aerobically for one day at 28 C on a rotary shaker (200 rpm).

Culture conditions

The yeast was cultivated in submerged culture with shaking in a Marconi MA-1550 Bioreactor. The medium was composed of the industrial residues molasses (2.5%), corn steep liquor (4.0%) and soybean oil (2.5%) supplemented with water. The pH was adjusted to

pH 5.5 with 1M HCl solution. The bioreactor (50 L) was filled with 20 L of liquid medium and sterilized at 121°C for 20 min. The inoculum was introduced in the amount of 10^4 cells/mL of the 24 h culture grown on YMB. The bioreactor was incubated at 28 C with shaking at 90 rpm for 144 h. The pH of the medium was not adjusted during cultivation. The efficiency of biosurfactant biosynthesis was evaluated in correlation with the doses of substrates used during the fermentation.

Stabilization of the biotensoative and determination of the surfactant's properties

The cell-free broth was centrifuged at 4500 rpm during 15 min, and submitted to a vacuum and paper filtration to remove the cells.

The stabilization occurred by fractioned tindalization for which autoclaving processes during three consecutive days, each one carried out day by day, were performed. The cell-free broth was then tested for its surfactant properties during 0, 15, 30, 45, 90, and 120 days. In each day, the pH was changed to 6, 8, and 10, 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, the emulsification and dispersion indexes were evaluated.

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.

The emulsification activity was measured using themethod described by Cooper and Goldenberg (1987), whereby 2 mL of an organic compound (soybean's oil, motor oil, or corn's oil) was added to 2 mL of the cell-free broth in a graduated screwcap test tube and vortexed at 7500 rpm for 2 min. The emulsion stability was determined after 24 h, and the emulsification index was calculated by dividing the measured height of the emulsion layer by the mixture's total height and multiplying by 100.

The dispersion index was carried out slowly by dropping of 15 µL of motor oil onto the surface of 100 mL of sea water layer (nearby to the cooling system of the energy generator from the Termelétrica de Pernambuco) contained in a Petri dish (14 cm in diameter) that spread all over the water surface area. This was followed with the addition of 1:1, 1:2, and 1:8 (v/v) of crude biosurfactant/ motor oil rate onto the surface of the oil layer. The average value of the diameters of the clear zones of triplicate experiments was measured and recorded then calculated as percentage of the Petri dish diameter (MORIKAWA et al, 2000).

RESULTS AND DISCUSSION

Stability of the biosurfactant properties under fractionated tindalization

Reductions in surface, and interfacial tensions are the mainly parameters to detect a tensoative compound (RAY, 2007). The properties like emulsification and dispersion are derivate from the surface tensions indicating that is essential to study all these features from a microbial surfactant (MUKHERJEE; DAS; SEN, 2006). In this paper, the surfactant properties were evaluated on each day (0, 15, 30, 45, 90, and 120 days) under different conditions of heating, pH, and salinity.

The best surface tensions were obtained after 30 days whose variation was between 26.69 until 30.73 mN/m, as showed in Figure 1. Furthermore, the majority of all surface tensions were minor then the normal surface tension of the biosurfactant (34.00 mN/m) reflecting that its stability is high. Moreover, the best conditions of the surfactant's properties were pH 6, 3% salinity and 50°C heating. It is important to say that if the conditions were applied together, it would probably occur the synergism's or antagonism's phenomena rising or decreasing the surfactant's properties.

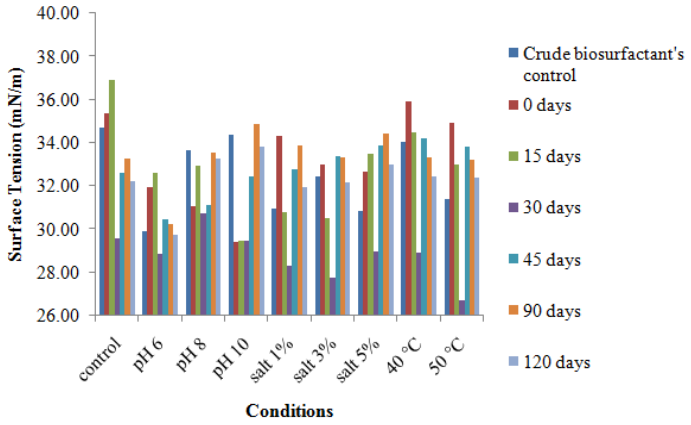

Figure 1 - Surface tensions from the biosurfactant produced in the bioreactor under fractionated tindalization during 120 days, measured in the control, heating, pH, and salinity conditions.

The emulsification's indexes were evaluated for three different oils: motor (A), soybean (B), and corn (C), as shown in Figure 2. The first oil presented the best emulsification indexesof 100%, and the best conditions at pH 6, 50°C heating and 5% salinity. In the soybean's and corn's oils the best conditions were respectively pH 10, 1% salinity and 40°C, and pH 10, 3% salinity and 50 °C.

Figure 3 displays results for the biosurfactant dispersion indexes (Figure 3). The rates (A) 1:1 (v/v), (B) 1:2 (v/v), and (C) 1:8 (v/v) have the lowest percentages except by the conditions at pH 8, and 10. The pH 8 was the best condition in all proportions to disperse the oil with the maximum percentage of 97.2%. Besides, the best salinities were 1, 5, and 3% in proportions 1:1, 1:2, and 1:8 (v/v), respectively, and the most efficient heating was at the temperature of 50 °C in proportions

1:2, and 1:8 (v/v), although in proportion 1:1 (v/v) the heating at 40 °C was better.

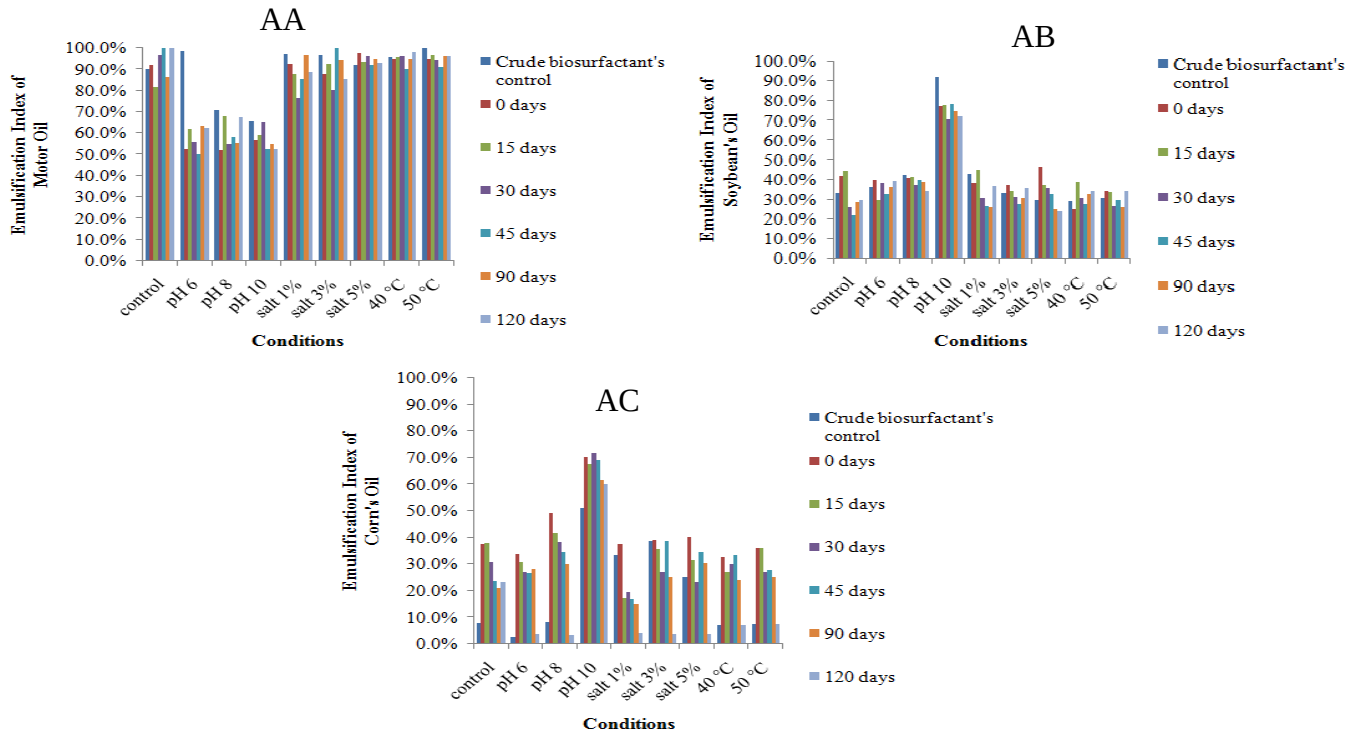

Figure 2. Emulsification's indexes from the biosurfactant produced in the bioreactor under fractionated tindalization, during 120 days, measured in the control, heating, pH, and salinity conditions in the oils: (A) motor, (B) soybean and (C) corn.

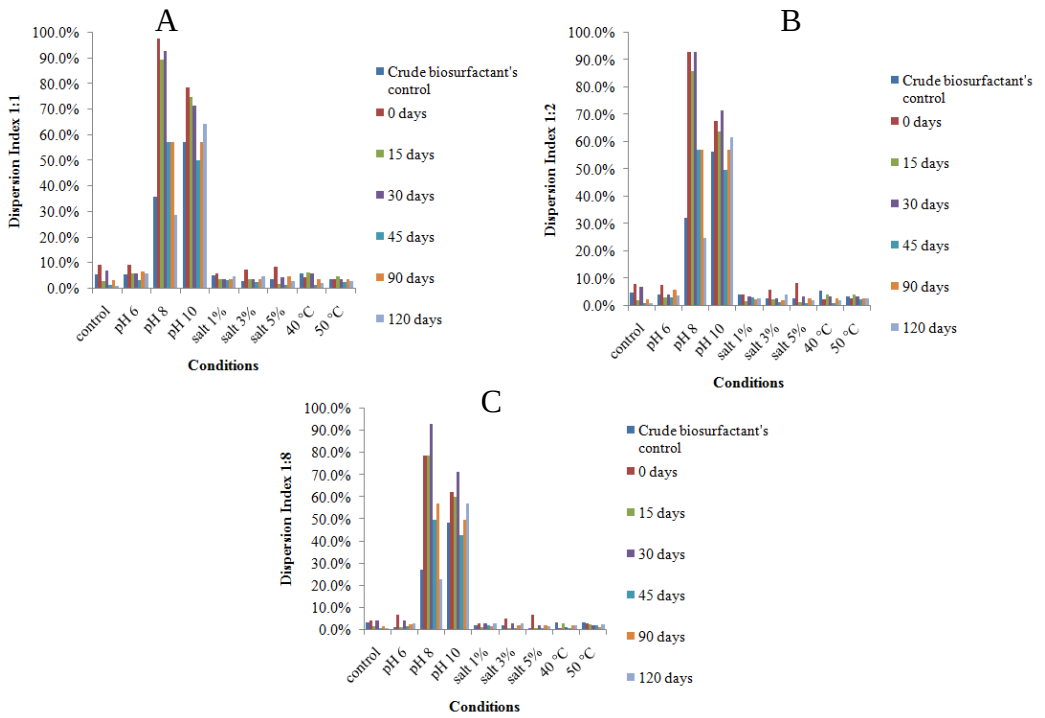

Figure 3 - Dispersion's indexes from the biosurfactant produced in the bioreactor under fractionated tindalization, during 120 days, measured in the control, heating, pH, and salinity conditions, and in the proportions: (A) 1:1, (B) 1:2 and (C) 1:8.

CONCLUSION

The surface tension could be reduced at about 7 mN/m reflecting the efficiency of the stabilization process.

The emulsification indexes were better in the motor oil mainly with the heating at the temperature of 50 °C , while the pH 10 was one essential factor in the soybean's and corn's oils emulsion.

The dispersion indexes were high only in hydrogenionic potentials 8, and 10 in the three proportions 1:1, 1:2, and 1:8.

The formulation of the biosurfactant was in ph 8, 3% salinity, or heating at 50 °C.

More research in biosurfactant's area should be developed to improve the application of this biomolecule.

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