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Application of Biosurfactant Obtained from *Eichhornia Crassipes* in the Removal of Petroderivate in Sand and Water from the Sea

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Oil spills and oil products destroy terrestrial and marine ecosystems. One solution to this is the use of biosurfactants. Biosurfactants are able to solubilize oil stains in soil and water. The intense reproduction of *Eichhornia crassipes* leads to a high density, causing obstructions in the waterway. The use of macrophytes for the production of biosurfactants represents the generation of value-added products. In this sense, roots of the plant were collected and they were submitted to several stages of extraction. The surface tension, the Critical Micellar Concentration (CMC) and the emulsification indexes were obtained for the extract. In addition, stability tests and applications of the vegetal extract were used to evaluate the removal of oil in sand and the dispersion of the oil in sea water. The plant extract was characterized by the presence of saponin, and the molecular structure of the biosurfactant was defined. The results showed a yield of 0.5 % of the vegetal extract, a surface tension of 27.57 mN/m and a high emulsification indexes of 65.4 %. Biosurfactant stability tests were performed at different pH, temperature and increasing concentrations of NaCl and they showed a little variation in the surface tension. The CMC of the vegetable biosurfactant was 1.4 g/L with a surface tension of 25.84 mN/m. The concentration of saponin was 1.29 g/L in the vegetal extract. The motor oil percentages removed from the sand by biosurfactant (CMC, 1/2 CMC and 2 times CMC), in the static test in packaged glass columns, were, respectively, 55.02, 57.41 and 68.04 %. The vegetable extract was also able to disperse 100 % of the motor oil into sea water. The biosurfactant was characterized as an unsaturated fatty acid. The tensoactive properties, the results of the petroderivative removal and dispersion experiments clearly demonstrate the feasibility of applying this new vegetable biosurfactant as a biotechnological additive to the remediation processes in environments affected by petroleum derived contaminants.

1. Introduction

The petroleum is an essential source of energy and it is an engine of economic development. According to the United States Department of Energy, 83 % of all primary energy sources consumed in the United States come from fossil source with petroleum that corresponds 57 %. In 2010, 19.2 million m^3 of petroleum was consumed per day. The USA produces 870,000 m^3 of crude oil per day from 530 thousand production wells, 35 % of them produce 0.16 m^3/day and 79 % produce < 1.59 m^3/day. The largest market for biosurfactants is in the oil industry, where they can be widely used in advanced oil recovery (MEOR - Microbial Enhanced Oil Recovery), in the removal and mobilization of oily residues, in bioremediation technology and in the removal of heavy metals (Almeida et al., 2016). Remediation of oil polluted areas has becoming a necessity, paving the way for the development of more ecological technologies to eliminate contaminants. The bioremediation is a set of technologies that allow the biodegradation of contaminants dispersed incorrectly in the environment. Biosurfactants have been successfully applied as remediation agents in aquatic and terrestrial environments,

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because it has the ability to solubilize hydrocarbons. The studies of biosurfactants started in 1960 and the use of these compounds has extended in the last decades as an attractive alternative to synthetic surfactants combined with environmental concerns and the new environmental control legislation (Silva et al., 2014).
The water hyacinth (*Eichhornia crassipes*) is an aquatic macrophyte that belong to the Pontederiaceae family. This specie has ecological importance because it has the ability to act as a biofilter, to accumulate heavy metals and to produce compounds of biotechnological interest (Almeida et al., 2015). Thus, considering the wide distribution of this species and its renewable character, the aim of this work was to investigate its tensoactive properties to use this macrophyte in the application of hydrophobic pollutants remediation.

2. Materials and Methods

2.1 Materials

The *Eichhornia crassipes* macrophytes were collected at the Apipucos Dam and the analyzes were performed at the Catholic University of Pernambuco, Brazil.

2.2 Extraction of Surfactants

The plants had their remnants of leaves, stems and roots separated. Then the roots were washed, dried and crushed similar to the method described by Wanyonyi, Onyari and Shiundu (2014). The surfactants were obtained by acid precipitation and solvent extraction (Abbasi et al., 2012).

2.3 Determination of the Tensoactive Properties and Stability of the Biosurfactant

The determination of the surface tension and the Critical Micellar Concentration (CMC) of the biosurfactant were determined by tensiometer, using the du Noüy ring. The emulsifying activity of the vegetable extract for different hydrophobic compounds (diesel, n-hexadecane, kerosene, motor oil, corn oil and soybean oil) were evaluated, according to Cooper and Goldenberg (1987). The effects of different environmental conditions (temperatures, NaCl concentrations, pH values and heating times at 90 °C) in the surfactant activities, which in turn were evaluated, according to Santos et al. (2018).

2.4 Saponin determination and nuclear magnetic resonance spectroscopy

The colorimetric method of saponin determination was performed by the method based on Hiai, Oura and Nakajima (1976). The purified biosurfactant was dissolved in deuterated chloroform (CDCl3) and the ^{1}H and ^{13}C NMR spectra were recorded under 27 °C using the Agilent 300 Mz spectrometer operating at 300 MHz. Chemical shifts (δ) were measured on the ppm scale relative to tetramethylsilane (TMS).

2.5 Dispersion test of the oil in sea water

The dispersion or aggregation capacity of petroleum by products was simulated in the laboratory by contaminating samples of distilled water with motor oil. The tests were conducted by adding of the formulated biosurfactant and motor oil in proportions (1:2, 1:8 and 1:25 v/v). The results were observed and measured visually (Saeki et al., 2009).

Figure 1: Glass columns used in the static test to remove engine oil impregnated in sand

2.6 Removal of motor oil from packed columns through static assay

The oil removability of the biosurfactant was evaluated using sand standard according Rufino et al. (2013). Glass columns 55 x 4 cm (height x diameter) were initially filled with a mixture of approximately 200 g of the soil containing 20 g of motor oil (15 cSt) (Figure 1). The surface was then inundated with 200 mL of the biosurfactant solutions isolated at ½xCMC (0.7 mg/L), 1xCMC (1.4 g/L) and 2xCMC (2.8 g/L), under the action of gravity. Percolation of the biosurfactant solution was monitored for 24 h until no further percolation of the solution was observed. The soil samples were washed with of hexane to remove residual oil and the amount of oil removed was determined by gravimetry.

3. Results and Discussion

3.1 Properties of the Biosurfactant-containing Plant Extract

The *Eichhornia crassipes* biosurfactant showed excellent results, with surface tension values lower than 25.00 mN/m in all evaluated conditions of pH (Figure 2a), salinity (Figure 2b), temperature (Figure 2c), and heating time at 90 °C (Figure 2d), demonstrating the high stability of the biotensoactive in the study. The Critical Micellar Concentration of the plant biosurfactant was 1.4 kg/m^3, and the surface tension was 25.84 mN/m at that point. The emulsification indexes obtained were promising for all oils, especially for motor oil reaching almost 70.0 % emulsification (Figure 2e). These results are within the emulsification index range of 32 % to 100 % found by Almeida et al. (2017) with the use of plant biosurfactants. In addition, emulsification activity are significant when compared to those reported by Lima et al. (2016), using *Eichhornia crassipes* (Mart.) collected from oil contaminated waters, to evaluate their potential for producing biosurfactants, obtained reduction of the surface tension for 51.03 mN/m and an emulsification index of 52.0 %.

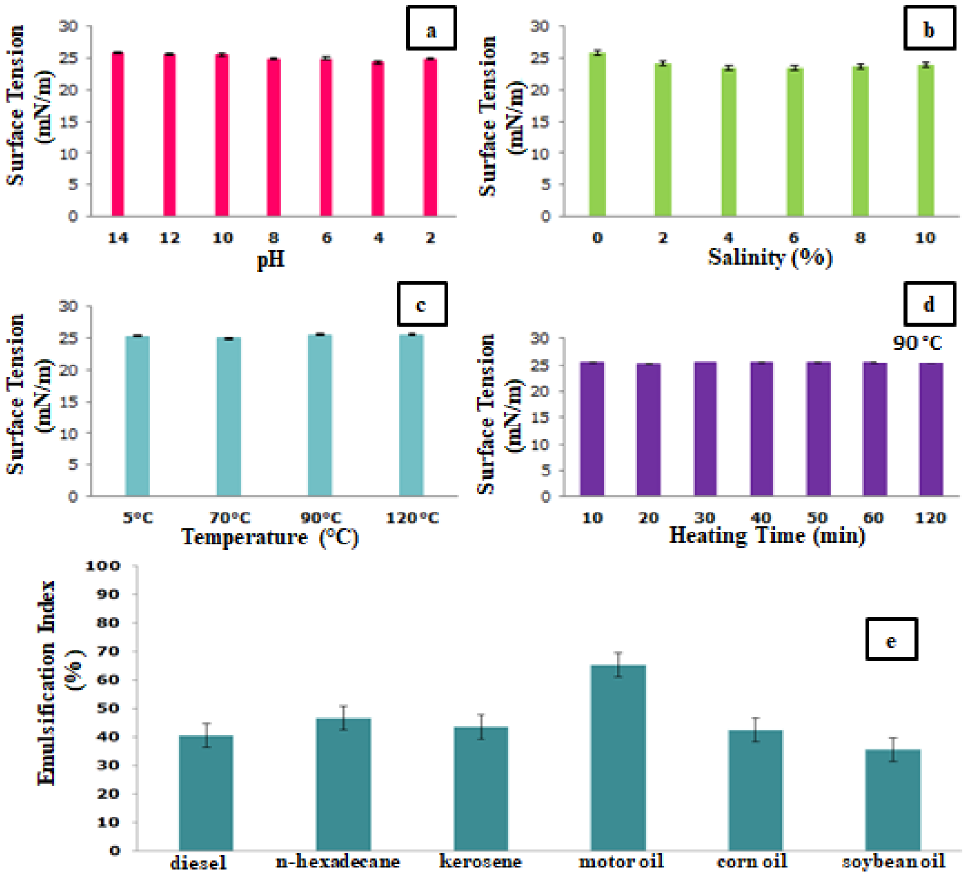

Figure 2: Stability of the biosurfactant of Eichhornia crassipes determined by the surface tension. (a) different pH values; (b) different concentrations of NaCl; (c) different temperatures, (d) temperature of 90 °C at different heating times and (e) Emulsification indexes of biosurfactant for diesel, n-hexadecane, kerosene, motor oil, corn oil and soybean oil

3.2 Characterization of the Biosurfactant

The result of the calibration curve is shown in Figure 3 along with the line equation and the coefficient of determination. The saponin concentration obtained from the line equation was 1.29 g/L in the plant extract. This value can be considered favorable compared to the concentration of saponin found in turmeric (*Crocus sativa*), which is between 1.2 and 3.4 g/L (Mir et al., 2016).

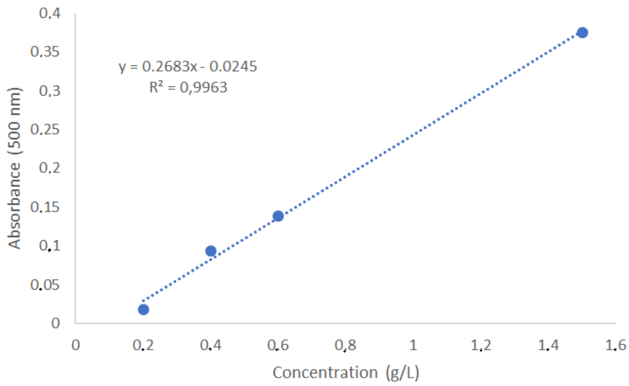

Figure 3: Calibration curve of the saponin standard along with the equation of the line and coefficient of determination

The composition of the biosurfactant obtained from the roots of *Eichhornia crassipes* was probed by ^{1}H NMR analysis (Figure 4). Signals between δ 0.55 and 1.7 ppm suggest the presence of methyl and aliphatic groups in the biosurfactant; between 1.8 and 2.1 ppm and those between 5.1 and 5.6 ppm indicate the presence of double bonds; between 2.2 and 2.5 ppm suggest the presence of carbonyl and those between 2.6 and 3.8 ppm correspond to carbon-linked hydroxyl. Signals at 0 and 7.25 ppm were assigned to tetramethylsilane and solvent residual signal (chloroform), respectively.

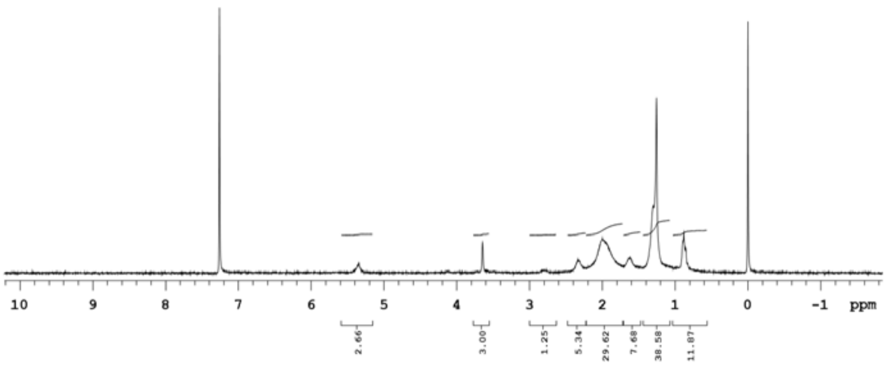

Figure 4: ^{1}H NMR Spectrum (CDCl3, 300 MHz) of biosurfactant isolated from Eichhornia crassipes roots

^{13}C NMR analysis according to Figure 5 shows signals from aliphatic carbons in the region between 12 and 38 ppm, hydroxyl-linked carbons at signals between 40 and 69 ppm, carboxylic acid between 176 and 179. Thus, biosurfactant extracted from the roots of *Eichhornia crassipes* is characterized by being a unsaturated fatty acid containing hydroxyl, similar to the abietic acid analyzed by Llevot et al. (2014).

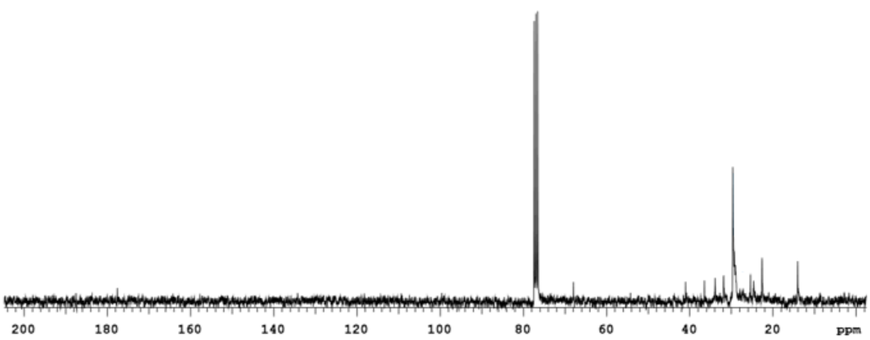

Figure 5: ^{13}C NMR Spectrum (CDCl 3, 300 MHz) of biosurfactant isolated from Eichhornia crassipes roots

3.3 Application of the Biosurfactant

The results for the plant biosurfactant obtained from *Eichhornia crassipes* showed that the surfactant was able to remove motor oil contained in sand from the packed columns (Table 1). Thus, the plant biotensoactive showed up to 68.04 % removal in 2xCMC. Bench scale laboratory studies using soils-packed columns are suitable to evaluate microbial recuperation of oil (MEOR) for several reasons: it is an economic model; a battery of columns can be set up simultaneously; and they can simulate the oil recovery operations usually conducted in reservoirs (Sarubbo et al., 2015). Studies carried out by Rufino et al. (2013) also using biosurfactants in packed columns demonstrated a maximum oil removal of 30 % from clay, using crude biosurfactant; and 33.1 % and 37.3 % from sand, using isolated biosurfactant concentrations at 1xCMC and 3xCMC, respectively.

Table 1: Rate of motor oil removed in sand by biosurfactant from Eichhornia crassipes in a half of the CMC, in the CMC and two times the CMC in static essay

Biosurfactant concentration (kg/m^3)	Rate of oil removed (%)
Control	35.08
1/2 x CMC	55.02
CMC	57.41
2 x CMC	68.04

The ability of a biosurfactant to disperse oils is extremely important in the treatment of environments contaminated by hydrocarbons, since this property accelerates the mobilization of oil by breaking the droplets, increasing the surface area of the oil in contact with degrading microorganisms (Freitas et al., 2016). The biosurfactant contained in the *E. crassipes* plant extract presented considerable results with percentages of 100 % of dispersion of oil stains in sea water similarly to crude bisurfactant from *Candida guilliermondii* UCP0992 studied by Sarubbo et al. (2016).

4. Conclusions

The tensoactive properties as well as the results of removal of the above mentioned petroderivates demonstrated the feasibility of applying this vegetable biosurfactant as an additive to the remediation processes. The plant extract showed significant concentration of saponin. *Eichhornia crassipes* biosurfactant is characterized as an unsaturated fatty acid containing hydroxyl.

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