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Evaluation of Emulsifier Stability of Biosurfactant Produced by *Saccharomyces lipolytica* CCT-0913

Álvaro Silva Lima[1*] **and Ranulfo Monte Alegre**[2]
[1]*Laboratório de Engenharia de Bioprocessos; Instituto de Ciência e Tecnologia; Universidade Tiradentes; Av. Murilo Dantas, 300; 49032-490; Aracaju - SE - Brasil. 2Departamento de Engenharia de Alimento; Universidade Estadual de Campinas; CP. 6168; 3083-970; Campinas - SP – Brasil*

ABSTRACT

Surface-active compounds of biological origin are widely used for many industries (cosmetic, food, petrochemical). The Saccharomyces lipolytica *CCT-0913 was able to grow and produce a biosurfactant on 5% (v/v) diesel-oil at pH 5.0 and 32°C. The cell-free broth emulsified and stabilized the oil-in-water emulsion through a first order kinetics. The results showed that the initial pH value and temperature influenced the emulsifier stability (ES), which was the time when oil was separated. The biosurfactant presented different stabilization properties for vegetable and mineral oil in water solution, despite the highest values of the ES occurring with vegetable oil. The biosurfactant presented smallest ES when compared to commercial surfactants; however, this biosurfactant was not purified.*

Keywords: Biosurfactant, emulsifier stability, fermentation, vegetable oil, diesel oil

INTRODUCTION

Surfactants are amphiphilic molecules consisting of a hydrophobic head and a hydrophobic tail. Due to their amphiphilic nature, surfactants can decrease surface and interfacial tension in water-oil, oil-water and oil-water systems (Cunha et al., 2004; Cameotra et al., 2003). Surfactants produced from chemically-based materials are known as synthetic surfactants and those from biologically-based material are biosurfactants. A wide variety of microorganisms present this ability as *Bacillus subtilis*, *Candida antartica*, *Acinetobacter radioresistensis* and *Serratia* sp (Urum and Pekdemir, 2004).

Biosurfactants are classified as ionic and non-ionic with varying chemical composition. They include glycolipids, lipopeptides, lipoproteins, phospholipids, fatty acids and polymeric surfactants (Youssef et al., 2004; Puntus et al., 2005). There are many advantages in replacing the industrial surfactants, which include: these compounds can be produced using relatively simple and inexpensive procedures and substrate. Some structural types of surfactants can be produced using the biological systems and are not easily synthesized by the chemical process. Biosurfactants are biodegradable, which is a positive ecological aspect. They are non-toxic or less toxic than the chemical surfactants. Surface activity molecules can be tailor made to suit different application by changing the growth substrate or growth conditions (Cameotra et al., 2004). These biological substances are used in therapeutic applications such as antibiotic and antifungical or antiviral compounds, in bioremediation of soil and sand and in the cleanup

* Author for correspondence: alvaro_lima@unit.br

of hydrocarbon contamination in groundwater (Youssef et al., 2004).

Biosurfactants may be characterized for their emulsifying properties. These properties are discussed in terms of emulsifying capacity (EC), emulsifying stability (ES) and emulsification activity (EA). The ES is commonly measured in terms of the time that an amount of oil and/or cream separating from an emulsion depending on temperature, gravitational field and the concentration of oil in the emulsion (Lima et al., 1997; Palejwala and Desai, 1989, Pearce and Kinsela, 1978).

The aim of this work was to study the influence of pH, temperature and biosurfactants type on emulsifier stability from diesel oil in water and the biosurfactant by *Saccharomyces lipolytica* CCT-0913 on emulsifier stability from vegetable oil and mineral oil in water.

MATERIALS AND METHODS

Materials

All chemicals were of reagent grade. The biosurfactants were supplied by Sigma Chemical Co., USA. Pectin was a polygalacturonic acid with high molecular weight. The majority of the structure consisted of homopolymeric partially methylated poly α(1→4)-D-galacturonic acid with present residues (rhamnogalacturonan, D-xylose, D-fucose, D-glucuronic acid, etc) (Pérez et al., 2003). Casein was composed by α, β and κ-casein and conjugated proteins (molecular weight of fractions is between 19,000 and 25,000). Tween 80® was a mixture composed of oleic, linoleic, palmitic and stearic acids. Sodium alginate was a sodium salt from brown algae (polyuronic acid composed primarily of anhydro-β-D-mannuronic acid residues with 1→4 linkage).

The vegetable oils used in this work were corn, sunflower, soybean, olive, dendê and canola oil. They were purchased in a supermarket in Campinas-SP-Brazil. The hydrocarbons used were benzene and kerosene.

Microorganisms and conditions of cultivation

Saccharomyces lipolytica CCT 0913 (actually *Yarrowia lipolytica)*, used in the present work, was obtained from André Tossello Foundation (Campinas-SP-Brazil). The microorganism was cultivated in 500 mL Erlenmeyer flasks containing 100 mL medium with the following composition (g/L): NaCl - 0.1, NH_4Cl - 5.0, KH_2PO_4 – 7.0, $MgSO_4.7H_2O$ – 0.4 and yeast extract – 1.0. The medium was supplemented with 5% (v/v) diesel oil as a sole carbon source. The pH initial value was adjusted to 5.0 using HCl 0.1 M and NaOH 0.1 M. Starter culture were grown for 24 h at 32°C in same medium. Flasks were inoculated (10%, v/v of the starter culture) and incubated on a rotary shaker (300 rpm) for 96 h at 32°C.

Emulsification activity (EA)

A known volume of broth was centrifuged at 6000 rpm and the aqueous phase was separated and filtered using a 0.45 μm membrane filter. Diesel oil (0.1 mL) was added to a sample (5 mL) of cell-free broth. The mixture was shaken for 2 min at room temperature. The resulting uniform emulsion was allowed to stand for 10 min, and its absorbance was measured at 540 nm every 10 min. The blank used contained the cell-free broth. One unit of emulsification activity was defined as the amount of biosurfactant that affected an emulsion with an absorbance at 540 nm of 1.0 (Cirigliano and Carman, 1984; Lima et al., 1997).

Emulsifier stability (ES)

Stability studies were done using cell-free broth in the different pH values (using 0.1 M NaOH and 0.1 M HCl) and temperature. The biosurfactant reduced the rate of emulsion decay. Supposing a first order kinetics,

$$\frac{d(EA)}{dt} = -k_d(EA) \qquad (01)$$

Where EA was the emulsification activity, t was the time (min) and k_d is the decay constant. Integrating the equation 01 between the time 0 and t:

$$\ln\left(\frac{EA}{EA_o}\right) = k_d \times t \qquad (02)$$

Where EA_o was the emulsification activity in initial time (t = 0 min).

The slope of the ln (EA/EA_o) *versus* t plot was a decay constant, which described the stabilization ability. As the smaller the k_d, the greater the stability was and then the relation showed in equation 03 could be used.

$$ES = -\frac{1}{k_d} \quad (03)$$

The emulsifier stability was measured with different vegetable oils and hydrocarbons using the cell-free broth, and several commercial surfactants (alginate, casein and pectin 0.75% w/v, and Tween 80® 0.75% v/v) using diesel oil.

RESULTS AND DISCUSSION

Effect of pH and temperature on emulsifier stability of biosurfactant by *Saccharomyces lipolytica*

The ability of the cell- free broth to emulsify and stabilize oil-in-water emulsion is shown in Fig. 1. It was observed that the emulsification activity decreased with the time (first order kinetics); each pH initial value reduced the rate of emulsion and produced different decay constants. In alkali initial pH (8-10), a precipitate formed, which interfered in emulsification activity and emulsifier stability determination as observed by other authors, when they obtained a polymeric biosurfactant (Cameron et al., 1998; Rosemberg et al., 1979). The initial pH value had an appreciable effect on emulsifier stability. At neutral pH the higher ES value (93.0 min in 40°C, pH 7.0 and 73.0 min in 50°C, pH 6.0) as shown in the Fig. 2 was observed.

The biosurfactant-containing solution was thermo-stable as shown in the Fig. 3. At high temperature, the higher emulsifier stability value (125.0 min in pH 6.0; 60°C and 99.0 min in pH 7.0; 60°C) was observed.

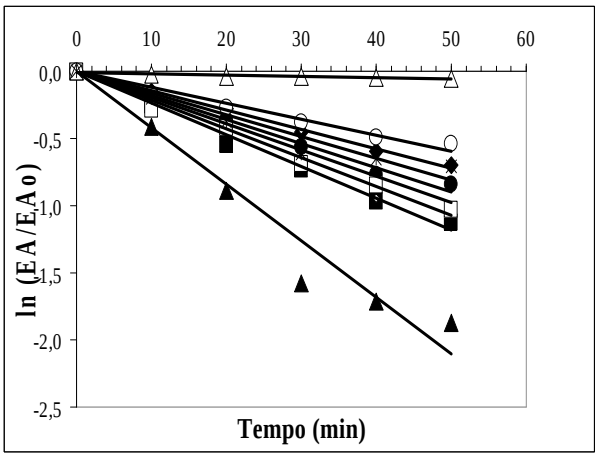

Figure 1 - Emulsifier stability biosurfactant by *Saccharomyces lipolytica* in oil-water emulsion at 30°C (◆- pH 2.0; ■- pH 3.0; ●- pH 4.0; ▲-pH 5.0; ◇- pH 6.0; □- pH 7.0; ○- pH 8.0; △- pH 9.0 and ∗- pH 10.0).

This stability was an important property of the biosurfactant produced by S *lipolytica*. Alasan from *Acinetobacter radioresistens* had heat stability and should facilitate the formation of sterile and stable oil-in-water emulsions (Navon-Venezia et al., 1995). Biosurfactants produced by *Bacillus subtilis* and *Norcardia* sp. was pH-stable (4.5 to 10 and 2-12, respectively) and thermo-stable (100°C) (Makkar and Cameotra, 1997; Kim et al., 2000).

Emulsifier stability biosurfactant by *Saccharomyces lipolytica* in oil-water emulsion

The biosurfactant by *S lipolytica* showed different stabilization properties in oil-in-water solution using commercial vegetable and mineral oil. The study was conduced in two cases (pH 6.0, 50°C and pH 7.0, 60°C). These conditions had been chosen to simulate the values applied in industries (higher temperatures and pH values next to the neutral zone).

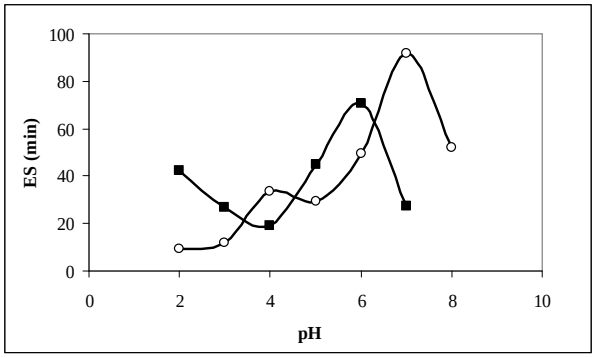

Figure 2 - Stabilization of diesel-oil emulsion by biosurfactant from *Saccharomyces lipolytica* in two temperatures (■- T= 50°C and ○- T= 40°C).

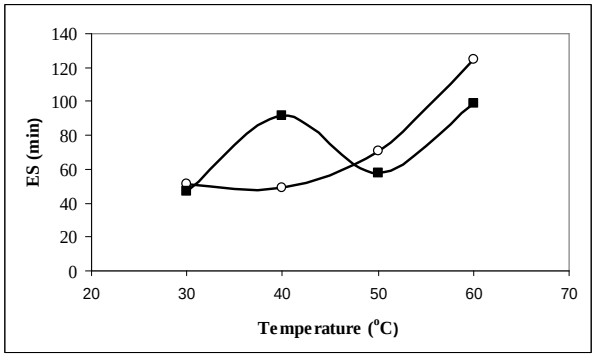

Figure 3 - Stabilization of diesel-oil emulsion by biosurfactant from *Saccharomyces lipolytica* in two pH values (■- pH = 7.0 and ○- pH = 6.0).

The results showed that the vegetable oils had higher ES than mineral oil, as shown in Figs. 4 and 5. *Rhodococcus rubber* produced an active surface substance when grown on individual n-alkanes; this biosurfactant formed stable emulsions of the *n*-hexadecane-water system (Kuyukina et al., 2005). This finding corroborated with the results reported for a biosurfactant by *Nocardia* sp. (Kim et al., 2000). The highest emulsifier stability was for pH 7.0 and 60°C, except for palm, kerosene, benzene and diesel oils, when compared to pH 6.0 and 50°C. The best ES values were found with olive oil (476.2 min pH 7.0 and 60°C) and dendê oil (263.2 min pH 6.0 and 50°C). Liposan by *C lipolytica* was most effective in stabilizing soybean (434.8 min), cottonseed (384.6 min) and corn (384.6 min) oils emulsions at pH 3,0 and 25°C (Cirigliano and Carman, 1985).

Comparison of emulsification and stabilization properties of biosurfactant and commercial surfactants

The ability of emulsification and stabilization properties of biosurfactants and commercial surfactants were studied using diesel oil as water-immiscible oil. The results are shown in the Table 1, and the surface activity compounds are ranked according to their stabilization properties in two conditions (pH 6.0, 50°C and pH 7.0 and 60°C).

The properties of the biosurfactant by *S lipolytica* are inferior to those commercial surfactants (88.5 min and 0.70 U at pH 6.0 and 50°C) and it ranked third at pH 7.0 and 60°C (99.0 min and 0.40 U), but this biosurfactant was not purified. Liposan showed an emulsification activity of 0.75U and an emulsifier stability of 166.7 min (Cirigliano and Carman, 1985), and *Nocardia* sp. produced an

emulsification and stabilization properties of 0.58 min^{-1} and 2.51 U, respectively (Kim et al., 2000). The results obtained in this work were sufficient to affirm that broth fermented could be use as a biosurfactant because have high emulsification activity and emulsifier stability for different condition of pH value, temperature and oil-water emulsions.

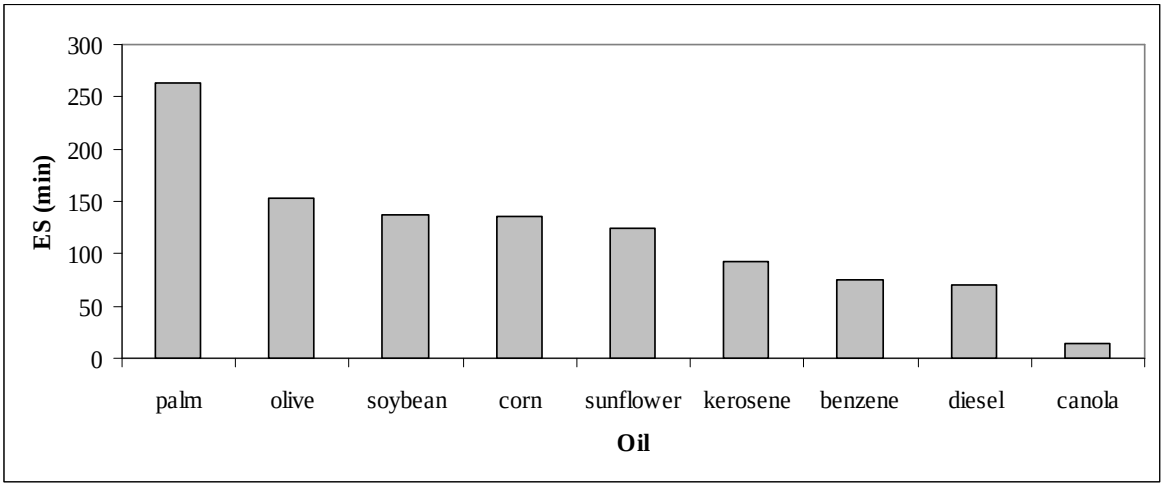

Figure 4 - Stabilization of oil-in-water emulsion by biosurfactant from *Saccharomyces lipolytica* in pH 6.0 and 50°C.

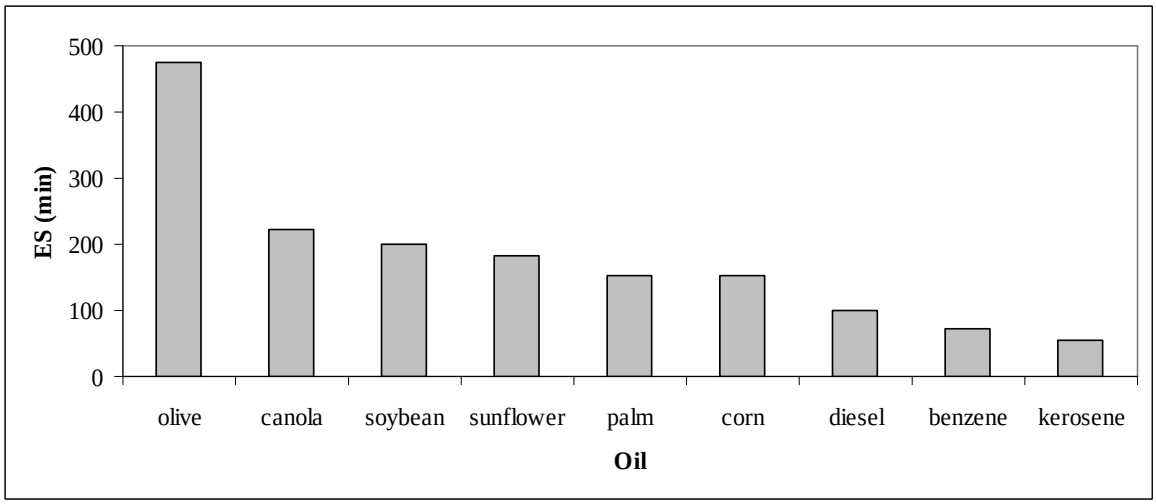

Figure 5 - Stabilization of oil-in-water emulsion by biosurfactant from *Saccharomyces lipolytica* in pH 7.0 and 60°C.

Table 1 - Comparison of emulsification activity and emulsifier stability of biosurfactant and commercial surfactants

	Experiment A		Experiment B	
Surfactant	**EA (U)**	**ES (min)**	**EA (U)**	**ES (min)**
Alginate	1.00	156.2	0.46	102.5
Pectin	0.99	98.0	0.53	151.5
Tween 80	0.89	108.7	0.78	92.6
Casein	0.45	107.5	0.14	75.2
Biosurfactant*	0.70	88.5	0.40	99.0

Experiment A: pH 6.0 and 50°C, Experiment B: pH 7.0 and 60°C. *-Biosurfactant produced in this work.

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RESUMO

Os tensoativos de origem biológica são amplamente utilizados em diversas aplicações. O microrganismo *Saccharomyces lipolytica* CCT-0913 possui a habilidade de crescer em 5% (v/v) óleo diesel a pH 5,0 e 32^{o}C e produzir biosurfactante. O caldo fermentado livre de células e produzido por *S. lipolytica* emulsiona e estabiliza emulsões óleo em água de acordo com uma cinética de primeira ordem. Os resultados mostram que o valor do pH inicial e a temperatura influenciam a estabilidade emulsificante (ES), que é medido pelo tempo que a quantidade de óleo. O biosurfactante apresenta diferentes valores de estabilidade emulsificante para óleos vegetais e minerais em emulsões óleo-água, os maiores valores de ES ocorrem nas emulsões utilizando óleo vegetal. O biosurfactante apresenta valores baixos de ES quando comparado com emulsificantes comerciais, entretanto sem sofrer nenhum processo de purificação.

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