

Commercial Formulation of Biosurfactant from Yeast and its Evaluation to Use in the Petroleum Industry

Darne G. Almeida^{a,c}, Rita de Cássia F. Soares da Silva^{a,c}, Pedro P. F. Brasileiro^{b,c},
 Juliana M. Luna^{b,c}, Raquel D. Rufino^{b,c}, Leonie A. Sarubbo^{*a,b,c}

^aNortheast Biotechnology Network, Federal Rural University of Pernambuco – RENORBIO/UFRPE, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos. CEP: 52171-900, Recife – Pernambuco, Brazil

^bCentre of Sciences and Technology, Catholic University of Pernambuco – UNICAP, Rua do Príncipe, n. 526, Boa Vista, CEP: 50050-900, Recife – Pernambuco, Brazil

^cAdvanced Institute of Technology and Innovation – IATI, Rua Carlos Porto Carreiro, n. 70, Boa Vista, CEP: 50070-090, Recife, Pernambuco, Brazil

leonie@unicap.br

Surfactants are amphipathic molecules capable of forming microemulsions of oil in water. Currently, the major market for surfactants is the petroleum industry. However, the limitations of these molecules to the extreme conditions encountered in the oil processing main stages have opened space for application of so-called biosurfactants, which are molecules more resistant, biodegradable and non-toxic. Biosurfactants are mainly produced by aerobic microorganisms growing in aqueous medium containing carbon and nitrogen source. These compounds act between fluids of different polarities (oil/water and water/oil), allowing access to hydrophobic substrates and causing a surface tension reduction, an increase in the oil contact area and an enhancement of the oil mobility and bioavailability and decreasing the viscosity. In this research, the application of a biosurfactant from *Candida tropicalis* UCP0996 in the formulation of a biodispersant was investigated. Cell-free broth obtained from *C. tropicalis* UCP0996 cultivated in industrial waste was mixed with an inexpensive and non-toxic preservative (0.2% potassium sorbate). The mixture was subjected to a long-term stability study to verify expiry date and recommend storage conditions, and an accelerated stability study to assess the impact of short exposure to adverse conditions outside those idealized for activity of the bioproduct. Properties of the formulated biosurfactant such as surface tension and emulsification were checked at 0, 15, 30, 45, 90 and 120 days. Then, formulated biosurfactant was examined about their dispersing efficiency against motor oil in seawater. As a result, formulated biosurfactant remained stable over time and under extreme conditions of pH, temperature and salinity. Moreover, the use of the formulated biosurfactant as biodispersant allowed reach levels of dispersion above 60 %. Therefore, this research allowed the formulation of a low cost biotechnological product with high durability that maintains the initial properties for many days, demonstrating its potential application in the oil industry as biodispersant.

1. Introduction

Petroleum is one of the major energy sources. The energy demand in the world indicates a 1.7% increase in the number of barrels of oil produced per year between 2000 and 2030, while consumption is expected to reach 15.3 billion tons of oil per year. Oil reserves allow meeting the world's demand for approximately 40 years if current levels of consumption are maintained. It is therefore important to develop technologies that allow the efficient use of this resource (Bachmann et al., 2014; CNI, 2007; EMBRAPA, 2006). Petroleum production is therefore also steadily moving toward unconventional crude oils including heavy/extra-heavy oils rather than medium to light oils, according to the International Energy Agency. In countries such as Canada, China, Mexico, Venezuela and the USA; the heavy and extra-heavy crude oils represents approximately half of recoverable oil resources. The development of efficient uses for this resource therefore is fast becoming an important technology (Cerón-Camacho et al., 2013).

Petroleum biotechnology has become an emerging technology that aims to implement biological processes to explore, produce, transform and refine petroleum to generate valuable by-products and to reduce, manage and clean any pollution output and to treat petroleum industrial effluents (Montiel et al., 2009; Silva et al., 2014). The versatility of microbes and microbial metabolism and their intrinsic ability to mediate transformation of complex raw materials at a wide range under extreme conditions such as high salinity, temperature, pH values, pressure and hydrophobicity, facilitates the development of these technologies (Montiel et al., 2009). Among the emerging biotechnologies with application prospects in the oil industry, those using biosurfactants have stood out promisingly (Silva et al., 2014). Biosurfactants have been applied effectively for the exploration of heavy oil, offering advantages over their synthetic counterparts throughout the entire petroleum processing chain (extraction, transportation and storage). Biosurfactants are used in microbial-enhanced oil recovery, the cleaning of contaminated vessels and to facilitate the transportation of heavy crude oil by pipeline (Assadi and Tabatabaee, 2010; Luna et al., 2012).

In the oil extraction process, the biosurfactant acts to reduce the surface tension of the oil-rock surface, reducing in turn the capillary forces that impede oil flow through the rock pores, helping to improve the oil recovery process from a depleted reservoir, thus prolonging the useful life of the container (Sarafzadeh et al., 2014). Crude oil has to be transported over long distances of extraction fields to refineries. This transport often causes operational difficulties that limit their economic viability. Among the main problems are low-flow, high viscosity and high content of asphaltenes and paraffins present in crude oil, which leads to problems of deposition and consequent drop in pressure, compromising the pipelines (Cerón-Camacho et al., 2013).

Various high molecular weight biosurfactants are powerful emulsifiers with outstanding ability to stabilize oil-in-water. Because of this ability, the biosurfactants this class has potential applications in the oil industry, promoting the formation of stable emulsions, helping reduce the viscosity during transport by pipeline (Assadi and Tabatabaee, 2010). Large quantities of crude oil are processed daily, distributed and placed in refinery storage tanks. The maintenance of these tanks require periodic washing, once waste and heavy oil fraction accumulate at the bottom and walls of the storage tanks and become difficult to remove solid deposits. The use of biosurfactants as an alternative cleaning procedure have become promising, since they can decrease the viscosity of the background deposits by formation of emulsion oil-in-water, thus facilitating the pumping of waste. Furthermore, this process allows the recovery of crude oil when the emulsion is broken (Matsui et al., 2012; Perfumo et al., 2010).

For all these applications, biosurfactant should be stable over time. The Resolution - RDC No. 45 of August 9, 2012 of National Health Surveillance Agency (ANVISA) provides for conducting stability studies of pharmaceutical ingredients assets. Based on this resolution, the stability studies adapted in this work were based on the following definitions: Long-term stability study - study designed to verify physical, chemical, biological and microbiological characteristics of a byproduct formulated, after period for expected validity. The results are used to establish or confirm expiry date and recommend storage conditions. Accelerated stability study - Study designed to accelerate possible chemical degradation or physical changes in bio-product formulated in conditions forced storage. The data thus obtained can be used to assess the impact of short exposure to adverse conditions outside those idealized for activity of the bio-product (RDC No. 45, ANVISA, 2012). Therefore, this study aimed to formulate a commercially stable biosurfactant obtained from *Candida tropicalis*, resistant to extreme environmental changes with the potential to be applied in the oil industry.

2. Materials and Methods

2.1 Materials

All chemicals were reagent grade. Growth media were purchased from Difco Laboratories (USA). Canola waste frying oil was obtained from a local restaurant in the city of Recife, state of Pernambuco, Brazil, stored according to the supplier's recommendations and used without any further processing. Corn steep liquor was obtained from Ingredion Brasil, Cabo de Santo Agostinho-PE, Brazil. Cane molasses was obtained from a local sugar mill in the municipality of Vitória de Santo Antão, state of Pernambuco, Brazil. Seawater was collected near the Thermoelectric TERMOPE, located in the municipality of Cabo de Santo Agostinho, in Pernambuco state, Brazil. Water samples were collected and stored in plastic bottles of 5 L.

2.2 Yeast strain and preparation of inoculum

A strain of *Candida tropicalis* UCP0996 was provided from the culture collection of the Catholic University of Pernambuco, Recife city, Pernambuco, Brazil. The microorganism was maintained at 5 °C on yeast mold agar 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 made to fresh agar slants each month to maintain viability. Inoculum was prepared by transferring cells grown on a slant to 500-mL Erlenmeyer flasks containing 100 mL of yeast mold broth (YMB). The cultivation conditions for the seed culture were 28 °C, 200 rpm and 24 h of incubation.

2.3 Formulation

The production of biosurfactant was performed in a basal medium composed of 2.5% cane molasses, 2.5 % waste frying oil and 2.5 % corn steep liquor in 500 mL Erlenmeyer flasks containing 100 mL of the medium and incubated with 2.0 % pre-inoculum and pH 5.5 with 200 rpm orbital shaking for 120 hours at 28 °C. After fermentation, the cell-free broth was submitted to a conservation method using 0.2 % of a preservative (potassium sorbate). After the treatment of the crude biosurfactant, broth was stored at room temperature (28–30 °C) for 120 days, with samples withdrawn at 15, 30, 45, 90, and 120 days (long term stability study). After each storage time, biosurfactant was subject to changes on pH (5.0, 7.0 and 9.0), addition of NaCl (1, 3 and 5 % w/v) and heating at 40 °C and 50 °C. Biosurfactant properties were checked by surface tension determination and emulsification activity in motor oil to verify the feasibility of the conservation method employed (accelerated stability study).

2.4 Determination of biosurfactant properties

Surface tension was determined with a Tensiometer (Sigma 700, KSV Instruments Ltd., Finland), using the Du Nouy ring method at room temperature (Silva et al. 2014). The emulsification index was determined using the method described by Cooper and Goldenberg (1987).

2.5 Application of biosurfactant formulated as dispersant

The oil displacement test was carried out by slowly dropping motor oil onto the surface of 40 mL of seawater in a Petri dish (150 mm in diameter) until covering the entire surface area of the Petri dish. This was followed by the addition of the biosurfactant formulated after had being stored for 0, 30, 45, 90 and 120 days, respectively and subject to changes on pH (5.0, 7.0 and 9.0), addition of NaCl (1, 3 and 5 % w/v) and heating at 40 °C and 50 °C, onto the surface of the oil layer in the proportions biosurfactant-to-oil ratio of 1:2 (v/v). Seawater was used as control. The mean diameter of the clear zones of triplicate experiments was measured and calculated as the rate of the Petri dish diameter (Ohno et al., 1993).

3. Results and Discussion

3.1. Formulation

One of the main requirements for byproduct formulation is that it should be stable over time and their properties should not significantly change with variations of pH, temperature, salinity, among others (Freitas et al., 2016). In this study, it was observed that through long term stability and accelerated stability studies it was found that the reduction of the surface tension properties of formulated biosurfactant remained practically constant over the 120 day test (Figures 1A – 1C). As can be seen in Figure 1, the inclusion of potassium sorbate on cell-free broth did not alter the properties of the biosurfactant, and the surface tensions at each storage period remained around 30 mN/m, equal to that found in the cell-free broth without sorbate (Batista et al., 2010). This confirming the application of potassium sorbate to maintain the biosurfactant produced by *Candida tropicalis* stable for a long time and extreme variations in the environment. Furthermore, the use of potassium sorbate dispensed with the additional cost of thermal treatment procedure, which made the process more economical from an industrial point of view.

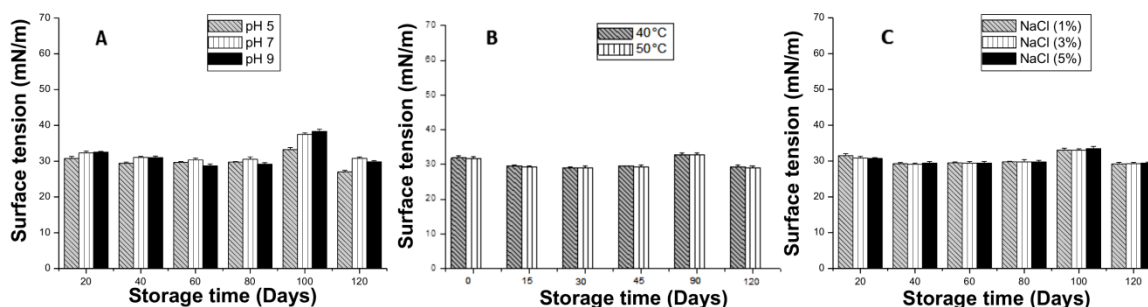


Figure 1: Surface tension of biosurfactant formulated with sorbate 0.2 % for 120 days under varying pH (A), temperature (B) and NaCl (C) after each storage period.

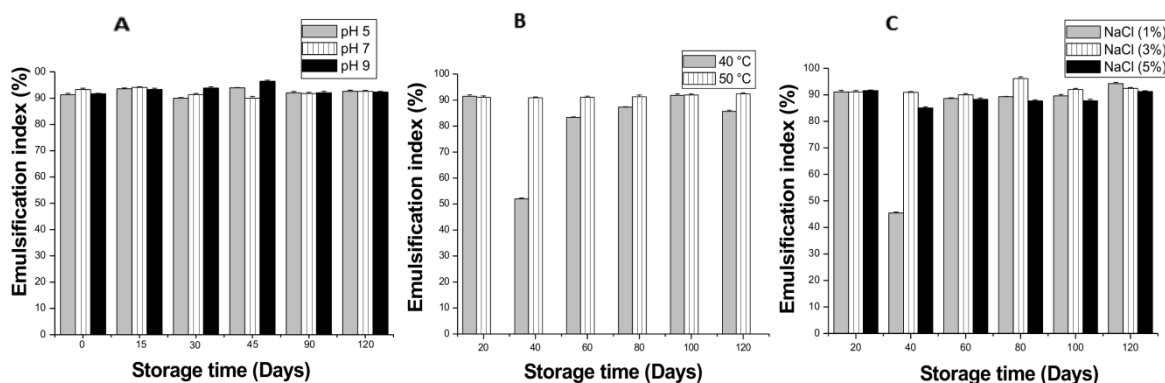


Figure 2: Emulsification index of biosurfactant formulated with sorbate 0.2% for 120 days under varying (A), pH, (B) temperature and (C) NaCl, after each storage period.

With respect to the property of emulsification it was observed that, generally, the formulated biosurfactant promoted a high rate of motor oil emulsification (above 80%) in virtually all conditions tested, with the exception of the condition where the temperature was equal to 40 °C and salinity of 1% at 15 days of test. Thus, for this type of oil, there was not practically change in emulsification index (Figures 2).

There are few studies related to the use of biosurfactants for formulation and implementation in several purposes, which makes this work more valuable contribution. Freitas et al. (2016) formulated a biodegradable commercial biosurfactant from *C. bombicola* URM 3718 cultivated in industrial waste for application as a dispersant in oil spills. Results obtained by them were also promising for the biosurfactant formulated with the preservative, which demonstrated stability through 120 days. For other applications, Campos et al (2015) tested six different formulations of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*. As a result, the most stable formulation with the best quality was obtained with combination of guar gum and the isolated biosurfactant with an absence of pathogenic microorganisms. In addition, the biosurfactant from *C. utilis* proved to be safe use in food emulsions. In another study, Bafghi et al (2012) studied the application of rhamnolipid in the formulation of a detergent. The results showed that the biosurfactant was effective in oil removal from the samples and the formulation presented was comparable to commercial powders in terms of the stain removal. Nguyen and Sabatini (2009) evaluated the formulating of alcohol-free micro-emulsions using rhamnolipid biosurfactant. They reported that the formulations obtained proved to be viable for a variety of applications. In another study, Youssef et al (2007) tested biosurfactant and synthetic surfactant mixtures in mobilizing of entrapped hydrocarbons. As a result, they obtained formulating biosurfactant mixtures that provided ultralow interfacial tension values against hydrocarbons.

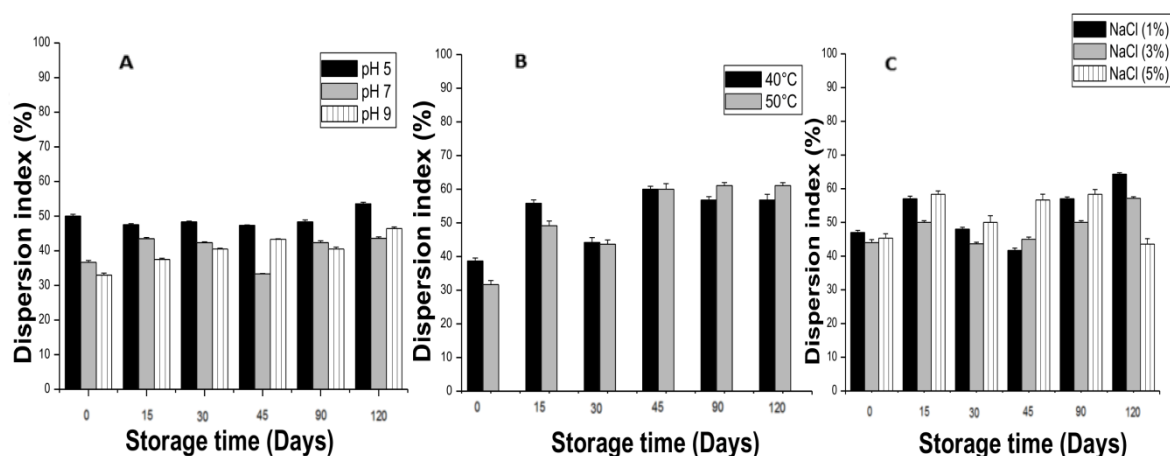


Figure 3: Evaluation of the biosurfactant formulated with sorbate 0.2% on the dispersion index under varying pH (A), temperature (B) and NaCl (C), after each storage period.

3.2. Application of biosurfactant as dispersant

Many processes carried out in the oil industry goes into the marine environment. Eventually, a part of the process oil accidentally reaches the seawater and, in turn, surfactants must be used in conjunction with other containment measures. In this study, it was evaluated dispersing ability of the formulated bioactive (Figure 3). As can be seen, dispersion indices were above 30 % in all conditions tested. At 50 °C, dispersion capacity of the formulated biosurfactant presented indices around 60 % between 45 and 120 experiments days (Figure 3B). The formulated biosurfactant was resistant to salinity variations, maintaining its dispersion capacity, which promoted 40 % dispersion of motor oil in the presence of salt (Figure 3C). Freitas et al. (2016) evaluated the dispersion capacity of a biosurfactant from the yeast *Candida bombicola* URM 3718 also formulated with the potassium sorbate addition. They obtained that potassium sorbate addition allowed dispersion between 30% and 50 % for the first 30 days of storage.

4. Conclusions

The greatest impact of this work resided in the formulation of a low cost biotechnological product with high durability that maintains the initial properties for many days exhibiting prolonged shelf life. Tests with the formulated biosurfactant revealed the feasibility of application without the need for purification of the product. In addition, formulated biosurfactant exhibited excellent stability under extreme environmental conditions of salinity, temperature and pH variations, demonstrating its potential for application in the oil industry as biodispersant.

Acknowledgments

This study was funded by the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE), the Research and Development Program from National Agency of Electrical Energy (ANEEL) and Electrical Central of Paraíba (EPASA), the National Council for Scientific and Technological Development (CNPq), and the Coordination for the Improvement of Higher Level Education Personnel (CAPES). The authors are grateful to the laboratories of the Centre for Sciences and Technology of the Universidade Católica de Pernambuco, Brazil.

References

- Assadi M., Tabatabaee M.S. 2010, Biosurfactants and their Use in Upgrading Petroleum Vacuum Distillation Residue: A Review, *Int. J. Environ. Res.* 4, 549-572.
- Bachmann R.T., Johnson A.C., Edye R.G.J. 2014, Biotechnology in the petroleum industry: An overview, *Int. Biodeter. Biodegr.* 86, 225-237.
- Bafghi M.K., Fazaelpoor M.H. 2012, Application of Rhamnolipid in the Formulation of a Detergent, *J. Surfactants Deterg.* 15, 6, 679-684.
- Batista R.M., Rufino R.D., Luna J.M., Souza J.E.G., Sarubbo L.A. 2010, Effect of medium components on the production of a biosurfactant from *Candida tropicalis* applied to the removal of hydrophobic contaminants in soil, *Water Environ. Res.* 82, 418–425. doi: 10.2175/106143009X12487095237279
- Campos J.M., Stamford T.L.M., Rufino R.D., Luna J.M., Stamford T.C.M., Sarubbo L.A. 2015, Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*, *Toxicol. Rep.* 2, 1164–1170.
- Cerón-Camacho R., Martínez-Palou R., Chávez-Gómez B., Cuéllar F., Bernal-Huicochea C., Clavel J.C., Aburto J. 2013, Synergistic effect of alkyl-O-glucoside and -cellobioside biosurfactants as effective emulsifiers of crude oil in water. A proposal for the transport of heavy crude oil by pipeline, *Fuel.* 110, 310–317.
- Cooper D.G., Goldenberg B.G. 1987, Surface active agents from two *Bacillus* species, *Appl. Environ. Microbiol.* 53, 224-229.
- CNI (Confederação Nacional da Indústria). 2007, Matriz energética: cenários, oportunidades e desafios. Publisher: CNI, Brasília, Brazil, 82.
- EMBRAPA. Plano Nacional de Agroenergia 2006 – 2011/Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Produção e Agroenergia, 2nd ed. rev.; Publisher: Embrapa Informação Tecnológica, Brasília, Brazil, 110.
- Freitas B.G., Brito J.G.M., Brasileiro P.P.F., Rufino R.D., Luna J.M., Santos V.A. Sarubbo L.A. 2016, Formulation of a commercial biosurfactant for application as a dispersant of petroleum and by-products spilled in oceans, *Front. Microbiol.* 7, 1646. DOI: 10.3389/fmicb.2016.01646
- Luna J.M., Rufino R.D., Campos-Takakia G.M., Sarubbo L.A. 2012, Properties of the biosurfactant produced by *Candida sphaerica* cultivated in low-cost substrates, *Chemical Engineering Transactions*, 27, 67–72, DOI: 10.3303/CET1227012

- Matsui T., Namihira T., Mitsuta T., Saeki H. 2012, Removal of oil tank bottom sludge by novel biosurfactant, JE1058BS, *J. Jpn. Pet. Inst.* 55, 138-141.
- Montiel C., Quintero R., Aburto J. 2009, Petroleum biotechnology: Technology trends for the future, *Afr. J. Biotechnol.* 8, 2653-2666.
- Morikawa M.H., Daido T., Takao S., Murata Y., Shimonishi, Imanaka T. 1993, A new lipopeptide biosurfactant produced by *Arthrobacter* sp. strain MIS38, *J. Bacteriol.* 175, 6459-6466.
- Nguyen T.T., Sabatini D.A. 2009, Formulating Alcohol-Free Microemulsions Using Rhamnolipid biosurfactant and rhamnolipid mixtures, *J. Surfactants Deterg.*, 12, 2, 109-115.
- Ohno H., Aoe T., Taki S., Kitamura D., Ishida Y., Rajewsky K., Saito T. 1993, Developmental and functional impairment of T cells in mice lacking CD3 zeta chains, *EMBO J.* 12, 4357-4366.
- Perfumo A., Rancich I., Banat I.M. 2010, "Possibilities and challenges for biosurfactants use in petroleum industry," in *Biosurfactants Advances in Experimental Medicine and Biology*, ed. R. Sen, (New York, NY: Springer), 135-145.
- Resolution - RDC No. 45 of August 9, 2012 of National Health Surveillance Agency (ANVISA).
- Sarafzadeh P., Niazi A., Oboodi V., Ravanbakhsh M., Hezave A.Z., Shahab Ayatollahi S. 2014, Investigating the efficiency of MEOR processes using *Enterobacter cloacae* and *Bacillus stearothermophilus* SUCPM#14 (biosurfactant-producing strains) in carbonated reservoirs, *J. Pet. Sci. Eng.* 113, 46-53.
- Silva R.C.F.S., Almeida D.G., Rufino R.D., Luna J.M., Santos V.A., Sarubbo L.A. 2014, Applications of biosurfactants in the petroleum industry and the remediation of oil spills, *Int. J. Mol. Sci.* 15, 12523-12542.
- Youssef N.H., Nguyen T., Sabatini D.A., McInerney M.J. 2007, Basis for formulating biosurfactant mixtures to achieve ultra low interfacial tension values against hydrocarbons, *J. Ind. Microbiol. Biotechnol.* 34, 497-507.