CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN 978-88-95608-56-3; **ISSN** 2283-9216

DOI: 10.3303/CET1864094

Production in Bioreactor and Application of Biosurfactant in Dissolved Air Flotation for the Treatment of Industrial Effluents

Juliana M. Luna[a,d*], Adriana A. L. Pinto[b], Maria Isabel S. Pinto[b], Pedro P.F. Brasileiro[c,d], Raquel D. Rufino[b,d], Darne G. Almeida[b,d] , Valdemir A. Santos[b,d], Leonie A. Sarubbo[b,d]

[a]Centre of Biological Scinces and Health, Catholic University of Pernambuco, Rua do Príncipe, n. 526, Boa Vista, Cep: 50050-900, Recife, Pernambuco, Brazil
[b]Centre of Science and Technology, Catholic University of Pernambuco, Recife, Pernambuco, Brazil
[c]Department of Chemical Engineering, Federal University of Pernambuco, Recife, Pernambuco, Brazil
[d]Advanced Institute of Technology and Innovation (IATI), Recife, Pernambuco, Brazil
Julianamouraluna@gmail.com

The oil industries are mainly responsible for the production of oily waters due to the drilling and extraction of petroleum, generating major environmental disturbances. The use of flotation as a separation process of oily waters has been described, although it has been sometimes criticized due to the toxicity of collectors. The development and use of biodegradable surfactants may enhance the further acceptance of this separation technology. Biosurfactants, molecules produced by microorganisms present as a sustainable and promising technology in increasing flotation efficiency by being biodegradable and non-toxic. It is also observed among the biosurfactants characteristics that promote the destabilization or stabilization of emulsions of the water-oil type, the first being the reason why some species can be used as auxiliaries of the flotation process. Thus, this study investigated the potential application of a biosurfactant in the separation of residual oil using a prototype of DAF. The biosurfactant was produced by *Candida guilliermondii* UCP 0992 grown in a low-cost medium and formulated with 4.0% of corn steep, 2.5% of molasses and 2.5% of soybean residual oil was carried out in a bioreactor 1.2 L at 28 °C for 144 h under 200 rpm. The operation in the prototype was conducted without addition of the biosurfactant and with addition of the biosurfactant in crude form using a central rotational compound design (DCCR), where the influence of the independent variables of effluent flow and biosurfactant flow on the response efficiency variable of separation. According to the results obtained, it was observed that the use of the biosurfactant increased from 40% to 94% the efficiency of the DAF process. The best result was 92% removal in the condition containing biosurfactant flow rate of 0.52 L/min and the effluent flow rate of 3.80 L/min. It is concluded that the use of biosurfactants as auxiliaries in the flotation is a promising alternative in the reduction of the pollution caused by the oily waters generated in the industrial environment.

1. Introduction

The continuous expansion of hydrocarbon processing industry and the extensive utilization of oil-related products in most industrial branches have increased the threat of oil pollution to the environment (Watcharasing et al., 2009). The use of flotation as a separation process, either as part of pollution control or during water treatment, has been sometimes criticized due to the likely toxicity of the collectors. The development and use of fully biodegradable surfactants may ease this concern and enhance the further acceptance of this separation technology (Menezes et al., 2011; Rubio et al., 2002). Surfactants are amphiphilic compounds that reduce the free energy of the system by replacing the bulk molecules of higher energy at a given interface. Surfactants have been used industrially as adhesive, flocculating, wetting and foaming agents, de-emulsifiers, and penetrants (Almeida et al., 2016; Santos et al., 2016). Considerable

Please cite this article as: Luna J., Pinto A.A., Pinto M.I., Brasileiro P., Rufino R., Almeida D., Dos Santos V. A., Sarubbo L., 2018, Production in bioreactor and application of biosurfactant in dissolved air flotation for the treatment of industrial effluents, Chemical Engineering Transactions, 64, 559-564 DOI: 10.3303/CET1864094

attention has been given to the production of surface-active molecules of biological origin to replace synthetic surfactants due to the advantages such as higher biodegradability, higher foaming, less toxicity, better environmental compatibility, more tolerant to pH, salt, and temperature variation, higher selectivity for metals and organic compounds and the ability to be synthesized from renewable feedstocks (Silva et al., 2016). Recently, novel surface-active biomolecules with attractive properties have been isolated from *Candida* species. These compounds have an anionic nature and are capable of reducing the surface tension of water from 70 to 28 mN/m, with a critical micelle concentration of 300 mg/L, which compares favorably with other known surface-active agents (Albuquerque et al., 2012; Sarubbo et al., 2015; Luna et al., 2017).
Thus, this study investigated the potential application of a biosurfactant as an alternative collector in the separation of residual oil using a prototype of the Dissolved Air flotation DAF.

2. Materials and Methods

2.1 Microorganism

Candida guilliermondii (UCP 0992) was obtained from the culture collection of the Catholic University of Pernambuco, Brazil. The micro-organism was maintained at 5 ºC 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 %).

2.2 Growth conditions

The inoculum of *C. guilliermondii a* was prepared by transferring cells grown on a slant to 50 mL of yeast mould broth. The seed culture was incubated for 24 h at 28 °C and agitated at 200 rpm. The inoculum (4% v/v) was introduced in the amount of 10^4 cells/ml to the cool production medium (Brasileiro et al., 2017).

2.3 Production of biosurfactant

The production of biosurfactant in bioreactor 1.2 L was performed in distilled water based medium with 4.0 % of corn steep liquor, 2.5% of molasses and 2.5% residual soybean oil. The medium was sterilised by autoclaving at 121 °C for 20 min (all components were sterilised together). The final pH of the medium was 5.5.

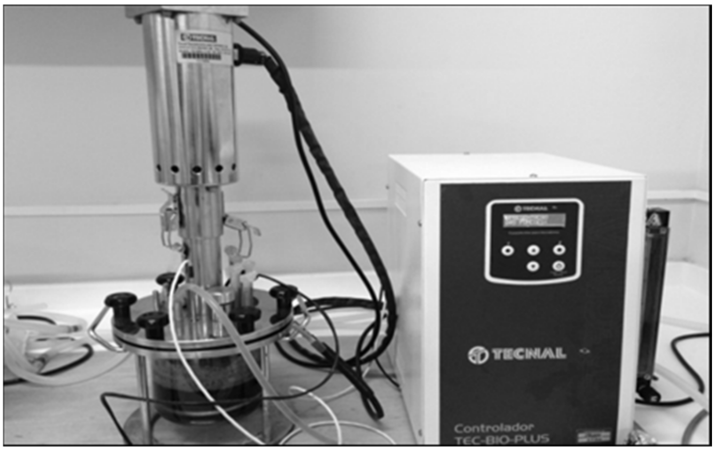

Figure 1: Biosurfactant production by C. guillermondii in bioreactor

2.4 Determination of surface tension

The determination of surface tension was carried out in the cell-free broth obtained by centrifuging the cultures at 5000 x g for 20 min at room temperature using a Sigma 700 digital surface tensiometer (KSV Instruments LTD - Finland), working on the principle of the *Du Nuoy* ring method.

2.5 Bench DAF Prototype

The oily water with 5 g/L of residual oil was subjected to treatment in a Dissolved Air Flotation (DAF) prototype (Rodrigues and Rubio, 2007) at a bench scale, as shown in Figure 2. The prototype was operated with the aid of a biosurfactant produced by the yeast *Candida guilliermondii*, as described by (Brasileiro et al., 2015) as a collector. As shown in Figure 1, a centrifugal pump (1) is responsible for feeding the oily aqueous phase stored in a tank (2). The flow of the oily aqueous phase to be treated passes through the flotation cell (3), with a volumetric flow rate adjusted by the opening of a valve (4). The control variables of the operating conditions of the system, such as levels and volumetric flow rates of the fluid, are recorded with the aid of an electronic circuit (5). Part of the treated water is sucked in by a centrifugal pump (6), saturated with air microbubbles. The dispersed oil droplets are surrounded by air microbubbles and float, giving rise to a kind of oily foam, which falls into a foam collector (7). A third centrifugal pump (8) sucks the remaining treated water and sends it for reuse. A Level Sensor Control System (9) keeps the prototype operating at steady state.

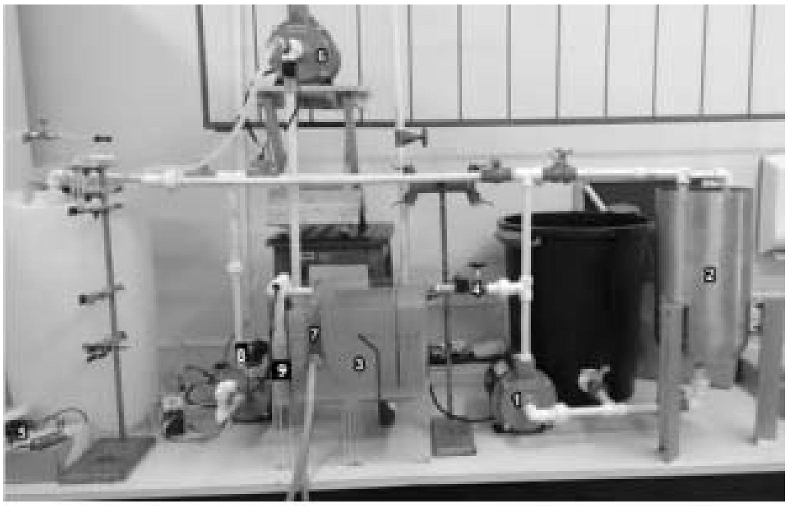

Figure 2: Bench DAF Prototype used for treating the oily aqueous phase

2.6 Removal of hydrophobic contaminant by flotation

The oil was extracted from the synthetic effluent samples with an equal volume of hexane (1:1, v/v). The mixture was vigorously shaken for 15 min and allowed to set until phase separation. The organic phase was removed and the operation was repeated twice. The product was concentrated from the pooled organic phases using a rotary evaporator. The amount of oil residing was gravimetrically determined (Lins et al., 2017)

2.7 Experimental Factorial Design and Response Surface Methodology

To analyze the effect of the use of biosurfactant on separation efficiency of the DAF system, the experiments were conducted according to CCRD. In order to establish optimal operational conditions of oil-water separation in the DAF system prototype, central composite rotational design applications were performed. Initially a CCRD like 2^2 was applied to define the operating conditions of the prototype. The independent variables were coded at five levels (-1.41, -1.00, 0.00, +1.00, +1.41) and the complete design consisted of 12 experimental point including 4 replications of the central points. The coded levels of the independent variables used are listed in Table 1.

Table 1: Experimental range and levels of independent variables for separation efficiency in DAF system with use of the biosurfactant

Tested Variables	Range and levels				
	-1.41	-1	0	+1	+1.41
Affluent Flow (l/min)	3.80	4.00	4.50	5.00	5.20
Biosurfactant Flow (l/min)	0.40	0.44	0.52	0.60	0.64

3. Results and Discussion

3.1 Central Composed Rotational Design (CCRD) Utilizing the Crude Biosurfactant

In the CCRD, both of the best removal results were at the assays 3 and 7 with biosurfactant flows of 0.60 and 0.52 L/min and affluent flows of 4.00 and 3.80 L/min, respectively, in the Table 2. Evidently, the use of the less surfactant flow is preferable, reflecting the assay 7 as the best. According to the results obtained, it was observed that the use of the biosurfactant increased from 40 to 94% the efficiency of the DAF process.

Table 2: Oil removal comparation between the variation of biosurfactant and affluent flows according to the CCRD

Assays	Biosurfactant Flow (l/min)	Affluent Flow (l/min)	Oil Removal (%)
1	0.44	4.00	66
2	0.44	5.00	64
3	0.60	4.00	94
4	0.60	5.00	87
5	0.40	4.50	62
6	0.64	4.50	90
7	0.52	3.80	92
8	0.52	5.20	83
9	0.52	4.50	82
10	0.52	4.50	80
11	0.52	4.50	84
12	0.52	4.50	82

After the analysis by the software Statistica for the CCRD, the equation 1 was formulated by the relation between the oil removal (OR), the biosurfactant flow (BF), and the affluent flow (AF).

$$OR(\%) = -51.66 + 850.17 \cdot BF - 550.78 \cdot BF^2 - 49.23 \cdot AF + 6.67 \cdot AF^2 - 31.25 \cdot BF \cdot AF \quad (1)$$

According to the equation 1, the best results were at the highest biosurfactant flows and at the lowest affluent flow applied as can be seen at the response surface, in Figure 3A, and at the contour surface, in the Figure 3B.

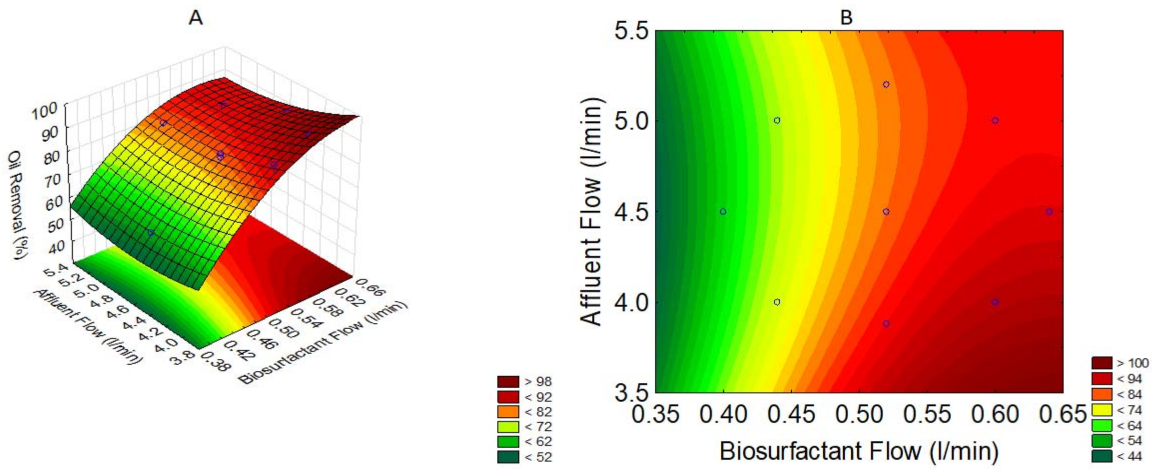

Figure 3: Oil removal view by the (A) surface response and (B) contour surface

In the Figure 4, the Pareto Diagram, for the quadratic model tested, all effects are statistically significant and are near 95% confidence level, confirming the information provided by ANOVA. These conditions of operation favorable to the indication of optimum conditions confirm the expected effects by Silva et al. (2015), since a biosurfactant and the same DAF prototype were used, with small adjustments in the physical structure of the experimental arrangement.

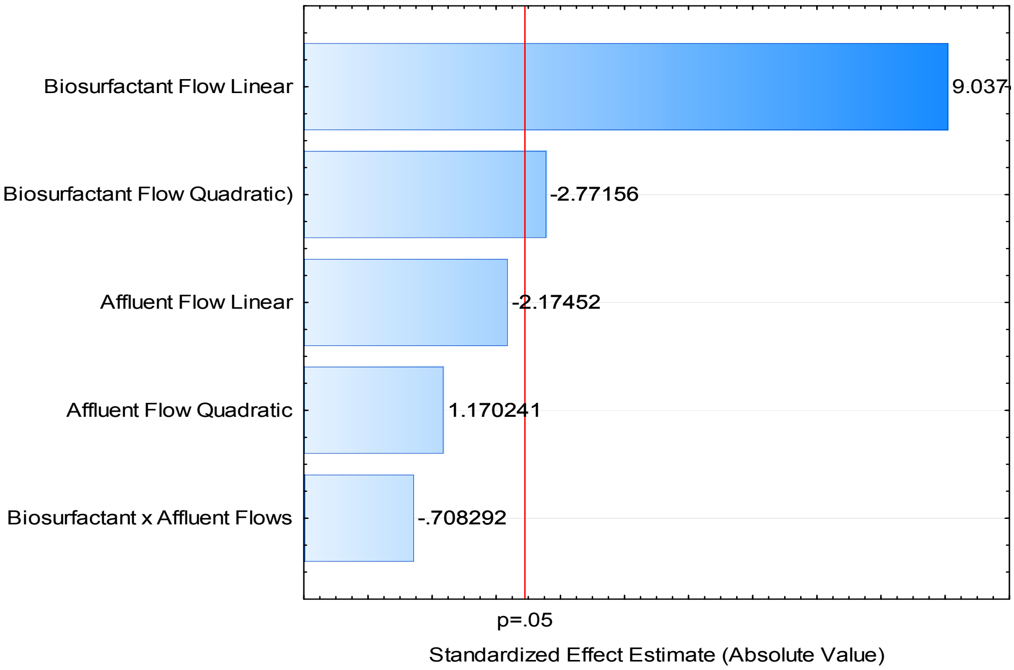

Figure 4: Pareto's Diagram of standardized effect for the biosurfactant and the affluent flows, utilizing the oil removal as response variable

3. Conclusions

The water-oil separation by means of the dissolved air flotation, applied with the aid of an experimental design of CCRD type, was an excellent methodology for the recovery of oily waters. The efficiency of the flotation process can be enhanced and ensured through the use of alternative collectors, such as surfactants of biological origin. Since biosurfactants are as effective in removing oil as synthetic collectors, the possibility of biosurfactants being recycled or degraded in ecosystems makes them attractive substitutes for synthetic surfactants in the treatment of wastewater and industrial effluents through flotation, there by contributing toward a reduction in environmental degradation and an improvement in the quality of water resources.

Acknowledgments

This study was funded by the Research and Development Program from National Agency of Electrical Energy (ANEEL) and Thermoelectric EPASA (Centrais Elétricas da Paraíba), the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE), 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 Centre of Sciences and Technology of the Universidade Católica de Pernambuco and to the Advanced Institute of Technology and Innovation (IATI), Brazil.

Reference

Albuquerque C.A., Luna- Finkler L.C., Rufino R.D., Luna J.M., Menezes C.T.B., Sarubbo L.A., 2012, Evolution of biosurfactant for removal metal ions from aqueous effuent using flotation techniques, *I.RE.CH.E*, 4,156–161.

Almeida D.G., Soares da Silva R.C.F., Rufino R.D., Luna J.M., Santos V.A., Banat I.M., Sarubbo L.A., 2016. Biosurfactants: promising molecules for petroleum biotechnology advances. Frontiers Microbiol. 7, 1718.

Brasileiro P. P. F., Roque B.A.C., Luna J. M., Rufino R. D., Santos V. A., Sarubbo L. A., 2017, The oil Cage: Arduino, biosurfactant and CCRD, Chemical Engineering Transactions, 57, 367-372.

Brasileiro P.P.F., Almeida D. G., Luna J. M., Rufino R. D., Santos V. A., Sarubbo L. A., 2015, Optimization of biosurfactant production from *Candida guiliermondii* using a Rotate Central Composed Design, Chemical Engineering Transactions, 43, 1411-1416.

Watcharasing S., Kongkowit W., Scamehorn S., 2009, Motor oil removal from water by continuous froth flotation using extended surfactant: Effects of air bubble parameters and surfactant concentration. Sep. Purif. Technology, 70, 179–189.

Lins J.M., Santos L.B., Santos, V.A., Sarubbo, L.A., 2017, Industrial reuse of water from chemical washing of residual frying oil, Chemical Engineering Transactions, 57, 523-528.

Luna J.M., Lima B.G.A., Pinto M.I.S., Brasileiro, P.P.F., Rufino R.D., Sarubbo, L.A., 2017, Application of *Candida sphaerica* biosurfactant for enhanced removal of motor oil from contaminated sand and seawater, Chemical Engineering Transactions, 57, 565-570.

Menezes C.T.B., Barros, E.C., Rufino R.D., Luna J.M., Sarubbo L.A., 2011, Replacing synthetic with microbial surfactants as collectors in the treatment of aqueous effluent produced by acid mine drainage using the dissolved air flotation technique. Appl. Biochem. Biotechnol. 163, 540–546.

Rodrigues, R.T., Rubio, J., 2007, DAF-dissolved air flotation: potential applications in the mining and mineral processing industry. Int. J. Miner. Process. 82, 1–13.

Rubio J., Souza M.L., Smith R.W., 2002, Overview of flotation as a wastewater treatment technique. Minerals Engineering, 15, 139–155.

Santos D.K.F., Rufino R.D., Luna J.M., Santos V.A., Sarubbo L.A. 2016, Biosurfactants: Multifunctional Biomolecules of the 21st Century. Int. J. Mol. Sci.17, 401.

Sarubbo L. A., Luna J. M., Rufino R. D., 2015, Application of a Biosurfactant Produced in Low-cost Substrates in the Removal of Hydrophobic Contaminants, Chem. Eng. Transactions, 43, 295-300.

Silva F.C.P.R.E., Silva N.M.P.R., Moura, A.E., Almeida R.G., Luna J.M., Rufino R.D., Santos V.A., Sarubbo L.A., 2015, Effect of biosurfactant addition in a pilot scale dissolved air flotation system. Sep. Sci.Technol. 50, 618-625.