CHEMICAL ENGINEERING TRANSACTIONS
VOL. 79, 2020

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Enrico Bardone, Antonio Marzocchella, Marco Bravi

ISBN 978-88-95608-77-8; **ISSN** 2283-9216

DOI: 10.3303/CET2079034

Treatment of Industrial Oily Effluent Using a Biossurfactant from *Pseudomonas Cepacia* CCT6659 as an Alternative Collector in a Bench Scale-Induced Saturation Tower

Aldemar Antônio P.C. Filho[a,c], Yslla Emanuelly da Silva Faccioli[a,c], Charles B.B. Farias[b,c], Pedro P.F. Brasileiro[a,c], Leonardo B.dos Santos[b,c], Valdemir A.dos Santos[a,c], Rita de Cássia F.S. da Silva[a,c], Leonie A. Sarubbo[a,c]

[a]Centre of Sciences and Technology, Catholic University of Pernambuco – UNICAP, Rua do Príncipe, n. 526, Boa Vista, CEP: 50050-900, Recife – Pernambuco, Brazil
[b]Northeast Biotechnology Network, Federal Rural University of Pernambuco – RENORBIO/UFRPE, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos. CEP: 52171-900, Recife – Pernambuco, Brazil
[c]Advanced Institute of Technology and Innovation – IATIg, Rua Joaquim de Brito, n. 216, Boa Vista, CEP: 50070-280, Recife, Pernambuco, Brazil
aldemarcamp@gmail.com

The increase in activities related to the oil and electric power sector are mainly responsible for the production of oily waters and their disposal, requiring treatment strategies and separation of these wastes. For the treatment of oily water, flotation technology employing air microbubbles for contaminant saturation can be employed efficiently. Chemical surfactants are applied as collectors for separation of suspended oil particles. Biosurfactants biodegradable and non-toxic, stand out as alternative collectors in the treatment of industrial effluents replacing synthetic and toxic surfactants. The present work aims to present an efficient solution through the of an innovative induced-air microbubble flotation prototype configured in a Stage Induced Saturation Tower (IST), in bench scale. This technology emerges as a more efficient and economical solution for the treatment of industrial wastewater using biosurfactants as collectors because they are biodegradable and have reduced toxicity. In this sense, the potential of the biosurfactant produced by *Pseudomonas cepacia* CCT6659 as an alternative collector in the treatment of an oily effluent from a thermoelectric plant was evaluated. Comparative tests were performed using the proposed biosurfactant, a cationic polymer, a synthetic surfactant (sodium dodecyl sulfate) and a commercial rhamnolipid classified biosurfactant of *Pseudomonas aeruginosa*. The tests were performed in the new system after homogenization between surfactant / effluent. The sample was fed at the top of the Tower and successively interacted with upward streams of air microbubbles at different stages. Samples of treated effluent were collected to evaluate the percentage of oil removal by spectrophotometry. The most significant results of the surfactant activities of the biosurfactant produced were: surface tension of 26 mN / m, emulsification capacity of 98.72% and dispersion action of approximately 86.00%. The IST system, with the action of microbubbles alone, achieved a 75.00% oil removal. The high efficiency of water / oil separation by *P. cepacia* biosurfactant compared to the collectors was proven from the removal of 97.49% of oil contained in the industrial effluent. Therefore, the potential of this surfactant agent as an alternative collector in an induced saturation flotation system proved helps to reduce the environmental impacts caused by industrial plants.

1. Introduction

Environmental problems have become increasingly routine, causing surface and groundwater pollution due to two major factors: population growth and industrial growth (Karlapudi et al., 2018). The increase in activities related to the petroleum and electricity sector are mainly responsible for the production of oily waters and their disposal, requiring systems for the treatment and separation of these wastes (Rocha e Silva et al., 2019). In this context, one of the main techniques of separation of water and oil that has been successfully highlighted

Paper Received: 31 August 2019; Revised: 4 December 2019; Accepted: 1 March 2020
Please cite this article as: Pedrosa Campello Filho A.A., Emannuelly Da Silva Faccioli Y., Bronzo Barbosa Farias C., Brasileiro P.P.F., Bandeira Dos Santos L., Dos Santos V.A., De Cássia Freire Soares Da Silva R., Asfora Sarubbo L., 2020, Treatment of Industrial Oily Effluent Using a Biossurfactant from Pseudomonas Cepacia Cct6659 as an Alternative Collector in a Bench Scale-induced Saturation Tower, Chemical Engineering Transactions, 79, 199-204 DOI:10.3303/CET2079034

in the treatment of effluents is the process of by dissolved air flotation (DAF), which consists of the separation of particles through adhesion of bubbles, allowing the reuse of the involved phases economically (Rocha e Silva et al., 2017; Albuquerque et al., 2012). However, the innovation presented in this work used induction air flotation for obtained more efficient. There is an increase in air contact with the effluent because it is a pro-current flow. Currently, horizontal floats with macro bubble action are used. However, the innovation presented in this work comprises a vertical float in stages with microbubble performance and probably nanobubbles. These microbubbles are more reactive because they reach a larger surface area, raising the separation efficiency.

The flotation uses mostly chemical surfactants to enhance the separation of the suspended material (Albuquerque et al., 2012). Surfactants are amphipathic molecules containing hydrophilic and hydrophobic moieties that partition at the oil / water or air / water interface. These characteristics allow surfactants to reduce surface and interfacial tension and form micro emulsions (Santos et al., 2016). In order to replace synthetic and toxic surfactants, some alternatives have been highlighted, such as the use of biosurfactants in flotation processes, because they are biodegradable and with low toxicity, mitigating environmental impacts (Almeida et al., 2016).

In the face of the challenges presented and the needs of development and improvement of the currently known techniques, this paper aims to propose effective solutions through technology of IAF. A new bench scale flotation system, configured in a stages saturation tower with the action of microbubbles, using a microbial biosurfactant as an alternative collector in the treatment and control of oily residues in the industrial area.

2. Materials and Methods

2.1 Synthetic Oily Effluent and Industrial Effluent

Knowing that 20 ppm is the maximum amount of oil allowed in industrial effluents in Brazil by CONAMA, the values of concentration of oil in water were established at 150 ppm. The effluent retired of a thermoelectric in the city of João Pessoa, state of Paraíba in Brasil has a composition of approximately 500 ppm (5 %) of heavy oil (OCB1 fuel oil, with a kinematic viscosity of 620 cSt, at most 1 % sulphur (p/p), a maximum of 2 % of water and sediment (v/v), flash point to 66 °C, fluidity point greater than 24-27 °C, maximum of 200 mg/kg of vanadium, PETROBRAS in Brazil) and a yellowish color resulting from the routine activities of the plant (Soares da Silva et al., 2018).

2.2 Commercial collectors

The water-soluble cationic polymer was used as a collector to increase the efficiency of the solid particulates in the industrial effluent in the concentration of (0.3 g / L). It is used as a flocculant in sewage treatment plants or industrial tributaries and is available commercially (FAXON QUÍMICA LTDA). Was used 2.4 ppm in the sodium dodecyl sulfate (SDS) according to Brasileiro, (2018). The product was supplied by the laboratory VETEC LTDA, Brazil. And a biosurfactant classified as a Raminolipid, supplied by the SIGMA-ALDRICH BRASIL laboratory, subsidiary of MERCK. The concentration used was ½ x CMC (150 mg / L).

2.3 Obtaining the Microbial Biosurfactant

The bacterium *Pseudomonas cepacia* CCT 6659, obtained from the culture bank of the André Tosello Research and Technology Foundation, located in the city of Campinas - São Paulo, was used as a microorganism producing the biosurfactant. Cultures were peaked every 30 days and kept in sloped test tubes with the solid Nutrient Agar (NA) medium under refrigeration at 5 °C. Young cultures of the bacteria were transferred to an Erlenmeyer containing 50 mL of BHI and maintained under 150 rpm orbital shaking for a period of 10-14 h at 28 °C to obtain a D.O. of 0.7 (corresponding to an inoculum of 10^7 U.F.C./mL) at 600 nm.

The production of biosurfactant was carried out according to Soares da Silva et al. (2017), used a mineral medium composed of: 0.5 g/L KH_2PO_4, 1 g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.1 g/L KCl and 0.01 g/L $FeSO_4.7H_2O$, supplemented with 2.0 % canola oil frying and residual 3.0 % corn steep liquor as substrates, pH 7.0, at a temperature of 28 °C, during 60 h of cultivation, under 250 rpm agitation and an inoculum of 1.5 %. The proposed biosurfactant is recognized by your excellent performance in bioremediation processes.

2.4 Formulation of biosurfactant

The cell free metabolic liquid containing the biosurfactant obtained after centrifugation for biomass removal was subjected to addition of 0.2 % potassium sorbate as preservative and conditioned under sterile conditions in a sealed container at room temperature (Soares da Silva et al., 2018).

2.5 Evaluation of the tensoactives properties of biosurfactant

The surface tension of the biosurfactant was measured on a KSV Sigma 700 (Finland) tensiometer using the NUOY ring. Subsequently the biosurfactant was extracted to determine his Critical Micellar Concentration (CMC) (Rocha e Silva et al., 2014). After extraction, the product was treated with a base and crystallized for maximum removal of impurities.

For the determination of the emulsification activity, samples of the formulated and isolated biosurfactant added in hydrophobic compound (motor oil) were analysed according to the methodology described by Cooper and Goldenberg (1987).

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

2.6 Evaluation of the biosurfactant as an alternative collector in the Tower of Induced Saturation (IST)

The tests were performed in an Induced Saturation Tower (IST) in Stages in bench scale, according to schematic representation (Figure 1). A 100 L volume of oily effluent added from the collector (crude biosurfactant *P. cepacia* (1.5 L), formulated biosurfactant *P. cepacia* (1.5 L), ½ x CMC isolated biosurfactant *P. cepacia* (300 mg / L) and commercial collectors in the established volumes), was homogenized for approximately 30 minutes to obtain a uniform distribution between water / oil / collector. Oily effluent without the addition of biosurfactant was used as a control. In this arrangement, the oily effluent was suctioned by pump 1 and recirculated in the tanks, the air was regulated with the aid of a 5.5 bar rotameter and a needle-type valve. The air enters interacting in current and, successively, forming upward streams of microbubbles in the bases of the stages 01 and 02. Then the pre-treated effluent falls in the acrylic tank where it will be suctioned by the pump 2, going to the stage 03 by the same process. Each has an effective volume of 3.4 L and operated with a nominal flow rate of 2.2 $L.h^{-1}$. The bubble particle aggregate formed was then transported to the upper section of each stage, due to the geometrical shape of the lid, exiting through a foam and solid collecting duct. The treated effluent was sent to the treated water tank. Samples of the treated effluent were collected after 5, 10, 15 and 20 minutes of process to evaluate the percentage of oil removal. Samples were evaluated by spectrophotometry; readings were taken at a wavelength of 330 nm using a calibration curve prepared with a standard 5000 mg / L oil solution. The results of this analysis allowed the calculation of the oil removal rate through the Equation: n = (Ci-Cf) / Cf * 100% (Campello Filho et al., 2019).

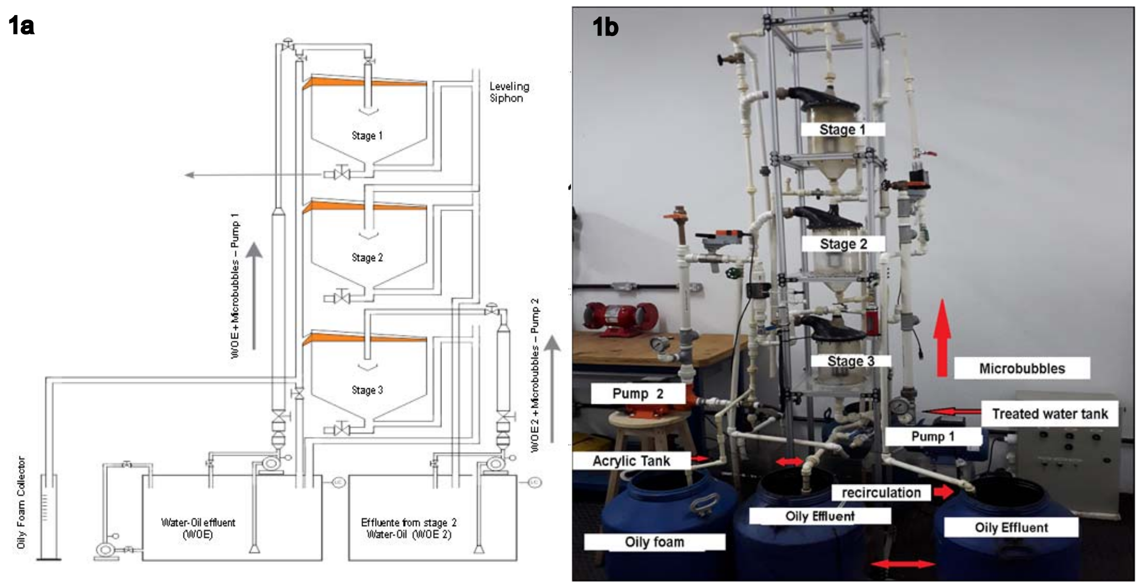

Figure 1 - Bank-scale induced saturation tower. (1a) indicates the flowchart and (1b) actual prototype photo

2.7 Statistical analysis of the data obtained

The data collected will be expressed as a mean ± standard deviation of the tests performed in quintuplicate. A statistical analysis of variance of ANOVA will be applied to determine a significance, where values of $p < 0.05$ will be analyzed.

3. Results and discussion

The majority of biosurfactants produced by bacteria have demonstrated the capacity to reduce surface tension to values around 28 mN/m (Soares da Silva *et al*., 2017). The formulated biosurfactant produced by *Pseudomonas cepacia* CCT 6659 was able to reduce the surface tension of the medium from 65 mN/m to 26 mN/m with a Critical Micellar Concentration (CMC) of 0.6 g/L.

The results of the emulsification activity demonstrated a high affinity of the microbial surfactant to the hydrophobic compound (motor oil) evaluated (Table 1). The formulated biosurfactant emulsified up to 95.5 % of the residual motor oil, demonstrating potential to be applied at oil contaminated sites. The ability to emulsify hydrocarbons depends on the hydrophobic compound as well as the structure of the biosurfactant, which is influenced by the substrate used. This capacity is related to the compatibility between the structure of the biosurfactant and the hydrocarbon, which will allow the stabilization or not of the microscopic droplets of the emulsion (Silva et al., 2014).

The dispersion percentages of the formulated and isolated biosurfactant demonstrated an optimum dispersant capacity of the surfactant evaluated (Table 1), with dispersion indices of approximately 75.0 %. It is evident that the biosurfactant produced by *P. cepacia* has stability in all the biosurfactant/motor oil relationships tested. The results indicate a good interaction between the biosurfactant and the motor oil employed, even at oil concentrations 25 times higher than that of the microbial surfactant.

Table 1 – Percentages of emulsification and dispersion of motor oil by Pseudomonas cepacia biosurfactant formulated and isolated

Biosurfactant / Oil	Formulated biosurfactant (%)	Isolated biosurfactant (CMC) (%)
Emulsification	95.50 ± 0.07	90.12 ± 0.12
Dispersion ½	75.23 ± 0.03	65.16 ± 0.14
Dispersion 1/8	53.22 ± 0.05	45.58 ± 0.11
Dispersion 1/25	40.47 ± 0.11	30.92 ± 0.17

The biosurfactant of P. cepacia in versions crude, formulated and isolated presented satisfactory results of oil removal in industrial effluent (Figure 2). It was observed that the assay without the addition of a collector, only with the action of microbubbles, showed maximum percentages of 75.0 % removal over all 20 minutes of testing. It is noteworthy that the tests with the addition of P. cepacia CCT6659 biosurfactant showed percentages of removal ascenders, as the residence time of the biocolector increased in relation to the oily effluent. Biosurfactant in crude and formulated versions achieved approximately 92.00 % removal and Isolate 97.49 % oil removal showing great stability during all 20 minutes of testing. Chemical collectors (sodium dodecyl sulfate (SDS) and cationic polymer) and commercial biosurfactant achieved percentages of less than 85.00 % removal during the same test time. Finally, the efficiency of oil removal in relation to the residence time of the tested biosurfactant should be studied. Campello Filho et al. (2019) investigated the separation of oil into water using FAI prototype, noting an increase in removal efficiency from 60 % to 80 % using biosurfactant. Silva et al. (2018) investigated the separation of oil into water using a pilot scale horizontal prototype (FAD) with and without the use of a microbial biosurfactant. The biosurfactant considerably improved the process, increasing from 41.0 % to 98.0 % in water / oil separation efficiency. Chaprão et al. (2018), also using a microbial biosurfactant in a horizontal prototype (FAD), achieved an oil removal rate of 92 % in a synthetic effluent. The results for the efficiency of oil removal in industrial effluent, through the tests performed by IST, proved excellent values, following the norms of the Brazilian environmental regulator (CONAMA).

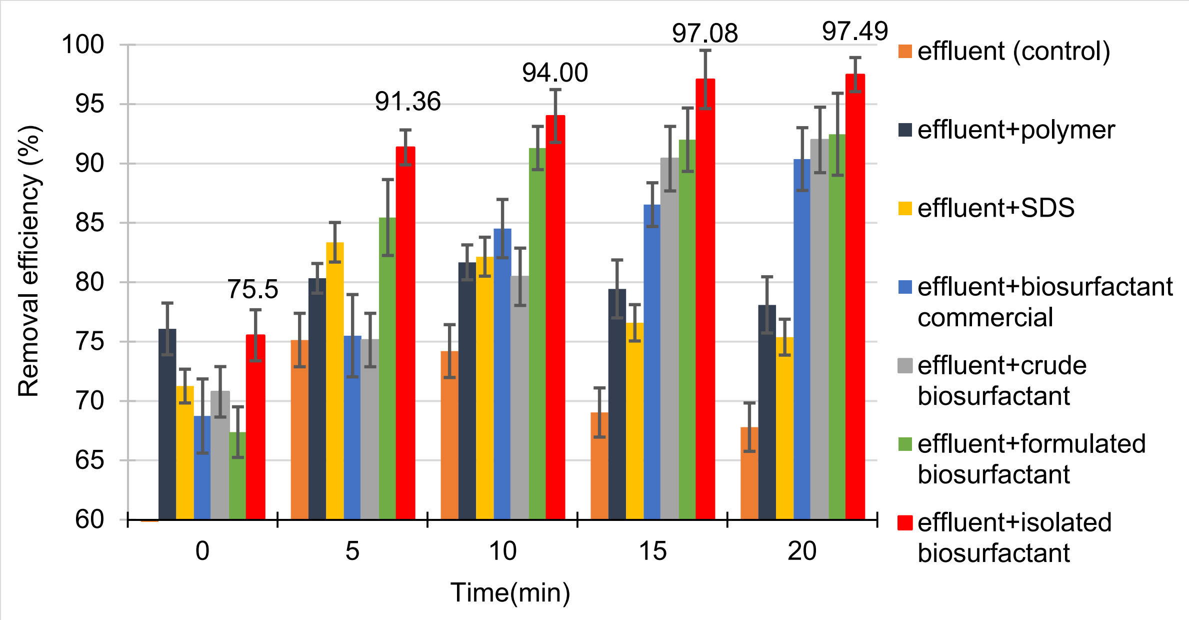

Figure 2 - Results of efficiency of oil removal in industrial effluent by Pseudomonas cepacia biosurfactant as collector compared to commercial collectors (SDS, polymer and commercial biosurfactant) associated with the action of microbubbles in the Induced Saturation Tower

4. Conclusions

The biosurfactant of *Pseudomonas cepacia* CCT 6659 presented excellent tensoactive properties and had its proven efficiency during its application in the Induction Saturation Tower (IST) on a bench scale compared to commercial collectors during the comparative study. In this way, the biotensoactive revealed great potential to be use as an alternative collector in the treatment of oily effluents in the IAF system, allowing an increase in the separation efficiency. Therefore, the innovative flotation system in staged, coupled with a biotechnological agent, demonstrated the ability to be applied in processes of separation of pollutants generated in industries plants.

Acknowledgments

This study was funded by the Research and Development Programme 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). Authors are grateful to the Centre of Sciences and Technology of the Catholic University of Pernambuco and to the Advanced Institute of Technology and Innovation (IATI), Brazil.

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