CHEMICAL ENGINEERING TRANSACTIONS

VOL. 74, 2019

A publication of AIDIC

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

Guest Editors: Sauro Pierucci, Jiří Jaromír Klemeš, Laura Piazza

ISBN 978-88-95608-71-6; **ISSN** 2283-9216

DOI: 10.3303/CET1974061

Biosurfactant from *Pseudomonas cepacia* CCT 6659 as Alternative Collector in Dissolved Air Flotation System for Soap and Detergent Industry Effluent Treatment

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A common practice adopted by several Brazilian companies is to collect residual frying oil and to use it for detergent or soap production. This practice, however, is challenging for the industry, since the use of waste oil generates a heterogenous effluent with high turbidity and containing numerous suspended particles. In this context, the flotation process has proven to be quite efficient for the treatment of this type of effluent, with the capability of removing a larger amount of oil in comparison to other methods. The development and use of microbiological surfactants may enhance even more the efficiency of this technology. These biological molecules also have several advantages over synthetic surfactants such as higher biodegradability, less toxicity, better environmental compatibility and can be synthesized from renewable feedstocks. The aim of the present study was to evaluate the separation efficiency of oil fraction from the effluent from the soap and detergent industry (ASA INDUSTRIA E COMÉRCIO LTDA, Recife – PE, Brazil) using a bench-scale DAF prototype with the addition of two different forms of a biosurfactant as alternative collectors. Biosurfactants was obtained from the bacterium *Pseudomonas cepacia* CCT 6659 and applied to the DAF system in both crude and isolated forms. The results demonstrated that the DAF-biosurfactant system increased oil separation efficiency of the DAF process from 69.49% (using only microbubbles) to 89.83 and 86.44% using the isolated and crude biosurfactant, respectively. In addition, there was also a turbidity reduction of the effluent, based on Brazilian Resolution CONAMA 430/2011. Therefore, the biosurfactant from *P. cepacia* in its isolated form was selected as the more promising product for the treatment of effluent from soap and detergent industry plant.

1. Introduction

The residual frying oil is part of one of the residues generated daily in homes, industries and public offices. Uncontrolled disposal of waste frying oils, in sinks or thrown directly into the water, entails a series of environmental damages, such as clogging of pipes in sewage systems, increased costs of treatment processes and increased pollution (Lins et al., 2017; Yu et al., 2017). The collection and reuse of these waste oils prevents their improper disposal and brings benefits to the environment. The percent recovery of residual oil from fried food depends largely on the washing operations. These operations are also responsible for the effective separation of the organic and aqueous emulsified waste phases, so that they have the lowest oil content, while the oil can be further used for the production of soaps and detergents (Lins et al., 2017; Silva et al., 2018). This practice, however, is challenging for the industry, since the use of waste oil generates a heterogenous effluent with high turbidity and containing numerous suspended particles. Besides that, effluent production of oily water type has generated many environmental problems for several industries (Yu et al., 2017).

Paper Received: 1 May 2018; Revised: 4 October 2018; Accepted: 28 January 2019

Please cite this article as: Almeida D., Luna J., Silva I.A., Silva E., Santos V., Brasileiro P., Sarubbo L., 2019, Biosurfactant from Pseudomonas Cepacia Cct 6659 as Alternative Collector in Dissolved Air Flotation System for Soap and Detergent Industry Effluent Treatment, Chemical Engineering Transactions, 74, 361-366 DOI:10.3303/CET1974061

Separation technologies such as centrifugation, ultrafiltration, decantation, flotation, and flocculation are examples of physical/chemical processes effectively used for the separation of oil-water mixtures (Painmanakul et al., 2010). However, the flotation process has proven to be the most efficient to clean this kind of effluent, with the capability of removing a larger amount of oil in comparison to other methods (Albuquerque et al., 2012; Silva et al., 2018). The use of flotation as a separation process of oily waters have been widely employed wastewater treatments, of oil industries (Bahadori et al., 2013; Rocha e Silva et al., 2015). The dissolved air flotation may be considered as a clean technology since it uses small quantities of coagulant and air to promote separation. The size, speed, and bubbles, along with the velocity gradient are important parameters to control the efficiency of the process and operating costs (Babaahmadi, 2010; Painmanakul et al., 2010; Szymanska and Sadowski, 2010).
The addition of coagulants/surfactants has favored the use of dissolved air flotation (DAF) in the removal of oil from wastewater, showing good results. Coagulants are used to improve the efficiency of separation and flotation of the oil droplets. On the other hand, this technique has been sometimes criticized due to the toxicity of this compounds used as collectors in this process (Menezes et al., 2011; Rocha e Silva et al., 2018).
Recently, the development and use of biodegradable surfactants has helped to increase acceptance of this separation technology. Biosurfactants are amphipathic molecules that reduce the surface and interfacial tensions of liquids. Such compounds have a predilection for interfaces of dissimilar polarities (liquid–oil) and are soluble in both organic (non-polar) and aqueous (polar) solvents (Almeida et al., 2016; Silva et al., 2014). These surface-active molecules of biological origin also have several advantages over synthetic surfactants such as higher biodegradability, higher foaming, less toxicity, better environmental compatibility, more tolerant to pH, salt, and temperature variation, and higher selectivity for metals and organic compounds and can be synthesized from renewable feedstocks (Almeida et al., 2017; Santos et al., 2016; Wang et al., 2013).
In order to obtain the biosurfactants for application in flotation processes, there is an infinity of microorganisms widely known in the world literature for its capacity to produce potent surfactants. These microorganisms can be grown in insoluble industrial residues (oily residues) and soluble (carbohydrates) in aqueous media, thus reducing production costs and the disposal of industrial waste in the environment (Freitas et al., 2016; Soares da Silva et al., 2018). Among the pioneering tests involving the DAF-biosurfactant system, we mention studies by Menezes et al. (2011), which demonstrated in a DAF operation for synthetic and biological surfactants tested under the same conditions, that the biosurfactant produced by *Candida lipolytica* presented superior results compared to sodium oleate, a chemical surfactant, for the removal of heavy metals. In addition, the biosurfactant reached lower values of turbidity, compared to the synthetic surfactant analyzed, than the limit established by Brazilian legislation. Albuquerque et al. (2012) also obtained, in a pioneering way, similar results for two biosurfactants tested, in comparison to the sodium oleate in the removal of heavy metals.
A number of other studies have also addressed the use of biosurfactant in flotation processes for effluents treatments. Two commercial surfactants, however, derived from microorganisms (Surfactin-105 and Lichenysin A) were used as collectors for the separation of metallic agents from effluents. The tests proved that biosurfactants showed better results than synthetic surfactants (SDS and dodecylamine) (Vecino et al., 2015). More recently, the biosurfactant of *Pseudomonas cepacia* was effective in the removal of oil from industrial effluents (Silva et al., 2018; Soares da Silva et al., 2017), which was also evaluated in the present study in the treatment of effluent from soap and detergent industry plant.
Thus, in this work, we investigated a water-oil separation by DAF, with addition of biosurfactant, in a pilot-scale DAF system. The experiments used to evaluate the effects of biosurfactant addition in DAF process also contributed to the assessment of whether aqueous phase can be reused in the industrial process itself.

2. Materials and Methods

2.1 Materials

All chemicals were of reagent grade. Growth media were purchased from Difco Laboratories, USA. Canola waste frying oil was received from a local restaurant in Recife-PE, Brazil and was stored according to supplier's recommendations and used without any further processing. Corn steep liquor was obtained from the Ingredion Brasil factory, Cabo de Santo Agostinho-PE, Brazil. The effluent used in this job was kindly provided by ASA INDUSTRIA E COMÉRCIO LTDA company, Recife - PE, Brazil.

2.2 Bacterial strain and inoculum preparation

A strain of *P. cepacia* CCT6659 was provided from the culture collection of the Fundação André Tosello de Pesquisa e Tecnologia, Campinas city, São Paulo, Brazil. The microorganism was maintained in nutrient agar slants at 4°C. For pre-culture, the strain from a 24-h culture on nutrient agar was transferred into 50 ml nutrient broth to prepare the seed culture. The cultivation condition for the seed culture was 28 °C, 200 rpm, and 24h of incubation time (Soares da Silva et al., 2013).

2.3 Biosurfactant production

The fermentation for the biosurfactant production was carried out in distilled water containing 2% of canola oil residual, 3% of corn steep liquor, 0.2% $NaNO_3$, 0.05% KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.01% KCl and 0.001% $FeSO_4$ $.7H_2O$. After media preparation, the pH was adjusted to 7.0 and these were autoclaved at 121°C for 20 minutes. The culture was incubated in a rotary Marconi MA832 shaker (Marconi Laboratory equipment, SP, Brazil) for 60 h at 200 rpm (Soares da Silva et al., 2017).

2.4 Surface tension and Critical Micelle Concentration (CMC) measurement

Surface tension was determined in the cell-free broth obtained by centrifuging the cultures at 10,000 × g for 15 min. Surface tension was determined with a Tensiometer (Sigma 700, KSV Instruments Ltd., Finland), using the Du Nouy ring method at room temperature. Surface tension and CMC measurement were determined in the isolated biosurfactant. CMC was determined by the surface tension obtained from frequent dilutions of the biosurfactant in Milli-Q water, until it reached a maximum surface tension in relation to surfactant molecules concentration. CMC result of the was obtained after stabilization of the concentration (Silva et al., 2014).

2.5 Isolation of biosurfactant

After cultivation, biosurfactant was recovered from the cell-free broth by cold acetone precipitation and isolated biosurfactant concentration was expressed in g/L, according to (Santos et al., 2017).

2.6 Evaluation of water-oil separation efficiency using biosurfactant as alternative collector

The effect of the biosurfactant in both isolated and crude forms on the separation efficiency was evaluated using a laboratory scale DAF unit (Figure 1), according to Silva et al. (2018). The pilot scale DAF system was constructed in clear and transparent acrylic to allow visualization of most of the fluid dynamic phenomena. The system was operated at ambient temperature (28 ºC) and pressure. The DAF chamber process the affluent, which comes into contact with the microbubbles. In chamber the oil droplets form the emulsion with the water coming into contact with air microbubbles and biosurfactant, yielding dispersed particles with lower average density than water, that are floated.

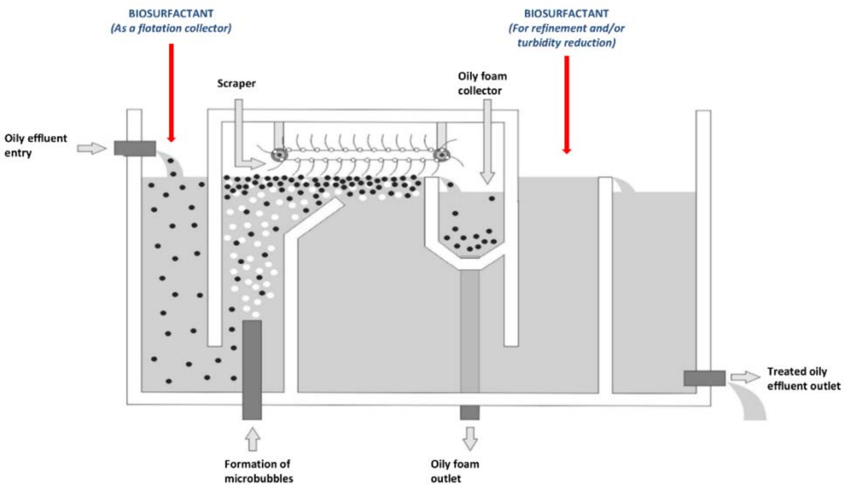

Figure 1: Bench-scale DAF prototype for treatment of the effluent from the soap and detergent industry

2.7 Hydrophobic contaminant removal by flotation

The oil was extracted from the 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 (Rocha e Silva et al., 2018).

2.8 Evaluation of the reduction of turbidity of the effluent by the biosurfactant

Effluent turbidity was monitored following the CONAMA Resolution 430/2011 (CONAMA, 2011). The turbidity meter (TB 1000, MS Tecno PON) was calibrated prior to the analysis, according to Silva et al. (2018).

2.9 Statistical analysis

ANOVA and the determination of regression coefficients were performed with the aid of the Statistica® program, version 12.0. All determinations were performed at least three times.

3. Results and Discussion

3.1 Biosurfactant production

The properties of the biosurfactant produced were verified prior to the tests for the study of its potential as an alternative collector. The biosurfactant produced was able to reduce the surface tension of the culture medium from 56.5 mN/m to 28.0 mN/m and CMC concentration of 600 mg/L. The data are supported by the results obtained by Silva et al. (2018) under similar conditions.

3.2 Evaluation of water-oil separation efficiency using biosurfactant as alternative collector

The efficiency of the treatment process was evaluated based on the removal of the oily fraction and turbidity of the effluent in the flotation system. Table 1 displays the results of the tests performed with the bench-scale DAF prototype and the isolated and crude forms of the biosurfactant from *P. cepacia* CCT 6659 applied to the effluent from ASA INDUSTRIA E COMÉRCIO LTDA company, Recife - PE, Brazil plant. The data indicate significant oil removal with both forms of the biosurfactant when compared to the process with the microbubbles alone. Few studies report the use of biological surfactants as collectors/coagulants in dissolved air flotation processes. One of the first studies conducted in Brazil on the development of DAF-biosurfactant systems for the treatment of oily water involved the use of a crude biosurfactant obtained from the yeast *Candida sphaerica* UCP 0995 cultivated in industrial waste during 6 days of fermentation. The authors reported an increase in the efficiency of separating oil from a synthetic effluent from 80.0% (using microbubbles alone) to 98.0% (in the presence of the biosurfactant) in a pilot-scale DAF system (Rocha e Silva et al., 2015). In a study conducted by Lins et al. (2017), a crude biosurfactant obtained from *C. lipolytica* UCP 0988 (cultivated for 3 days using industrial waste as the substrate) led to an improvement in oil removal from an actual effluent comprised of waste frying oil in a pilot DAF system, achieving an oil separation rate of 95.5%. In a more recent study, Luna et al. (2018) applied a crude biosurfactant from *C. guilliermondii* UCP 0992 in the treatment of a synthetic oily effluent using a bench-scale DAF system, achieving an increase in the removal efficiency from 40% (microbubbles alone) to 92% with the crude biosurfactant. The studied DAF system with the use of biosurfactant as adjuvant in the process presented a better performance. In the present study, biosurfactants obtained in a rapid-growth fermentative medium (2–2.5 days) were used rather than long-growth media for yeasts (3–5 days), the biosurfactants of which have previously been employed more in innovative DAF-biosurfactant systems for the treatment of oily water (Freitas et al., 2016; Santos et al., 2017).

Table 1: Oil removal rates using flotation process and total turbidity of oily effluent from soap and detergent industry (ASA INDUSTRIA E COMÉRCIO LTDA company) using the biosurfactant from P.cepacia CCT 6659 in formulated and isolated forms as alternative collector

Conditions	Removal of oily fraction (%)	Turbidity (NTU)
Oily effluent (control)	-	917.84
Microbubbles alone	69.49	394.17
Crude biosurfactant from *P.cepacia* CCT 6659	86.44	417.47
Isolated biosurfactant from *P.cepacia* CCT 6659	89.83	253.01

The turbidity of the effluent was also verified. As can be seen (Table 1), the effluent underwent turbidity reduction with the microbubble process, and then a slight increase using the biosurfactant, most likely due to the staining of the cell-free metabolic fluid used. With the biosurfactant there was a reduction of almost 72.43% in the turbidity in relation to the turbidity of the initial effluent. With the process employing only microbubbles, there was a reduction of 57.05%. The results showed that the biosurfactant of *P. cepacia* CCT 6659, in its isolated form, was the most promising for the flotation process using the ASA effluent, achieving a good removal of the oil fraction and an excellent reduction of the turbidity of the system. In addition, it was possible to conclude that the use of biosurfactants in their crude form, although decreasing the cost of insulation/refinement processes, presents costs related to the conservation of the product during transport and storage, being necessary its production *in loco* (in the own plant), demanding additional structure for such goal (Silva et al., 2018). On the other hand, the isolated biosurfactant, although it has significant costs associated with the biomolecule insulation, can be produced outside the plant environment, and be transported and stored with more practicality (Soares da Silva et al., 2018). Thus, the biosurfactants in the isolated form demonstrate greater potential for technical and economic feasibility, when evaluated its application in the DAF process. The use of industrial waste products in the production of the biosurfactant is in line with current concerns for sustainable practices. The treatment of industrial effluents and oily water using the DAF–biosurfactant system developed herein can contribute to a reduction in environmental degradation and the improvement in the quality of water resources. Therefore, the present study describes a favourable, efficient strategy for the decontamination of oily industrial effluents (Chaprão et al., 2018).

4. Conclusions

The present study demonstrated the effectiveness of using a central composite rotational design to identify the optimum parameters for increase oil removal efficiency in DAF system. The above results confirm the great potential of the biosurfactant to be used as an alternative collector, since these microbial surfactants act as true "molecular glues", interacting with the oil and the air bubbles, facilitating the oil transportation during the process flotation, as evidenced by the results presented.

Acknowledgments

This study was funded by the Research and Development Program from National Agency of Electrical Energy (ANEEL) the Candeias Energy Company (CEC) from Global Group, through the Project code PD-06961-0005/2016, 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.

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