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Application of a Biosurfactant as a Collector in Flotation Chamber with Induced Pre-saturation in Bench Scale for Oil Water Treatment

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In the current scenario, one of the major environmental problems is the treatment of oily water generated during industrial activities. Flotation employing the saturation of the effluent with air microbubbles is a very promising technique, due to its efficiency and control condition of physical variables, such as: size of the microbubbles, hydraulic retention time and the contaminant concentration in the effluent. The remediation process of contaminated areas has been driving the market towards the development and use of biodegradable surfactants, which act as alternative collectors in flotation and they is being able to increase even more the acceptance of this separation technology. The objective of the present study was to apply a biosurfactant of *Bacillus methylotrophicus*, in crude, formulated and isolated versions in a flotation chamber with induced pre-saturation, on a bench scale, in comparison with other commercial collectors for the treatment of an oily synthetic effluent. The results indicated that the commercial biosurfactant reached 90.52% removal and the chemical surfactant sodium dodecyl sulfate obtained 86.74% removal. The biosurfactant of *B. methylotrophicus* demonstrated ascending percentages of removal as the collector residence time increases in relation to the effluent. The crude biosurfactant reached 98.0% of removal, the formulated biosurfactant reached 98.39% of removal and the isolated biosurfactant reached 99.0% of oil separation, demonstrating the potential of application as a collector of oily contaminants in industrial processes.

1. Introduction

In the last decades, due to increased industrial activities, environmental problems have become growing routine, causing the pollution of surface and groundwater due to two major factors: population growth and industrial growth (Cai et al., 2018). The use of flotation as a separation process has sometimes been criticized due to the probable toxicity of the collectors, considering that chemical surfactants are used as collectors in the separation process of oil in water (Menezes et al., 2011).

The presence of oils does not indicate the only determinant of toxicity in the environment, evidence linking the greater presence of polycyclic aromatic hydrocarbons in oils dispersed by chemical surfactants present a greater toxicity to aquatic organisms. Some alternatives have been highlighted, such as the use of biosurfactants as they are biodegradable and are known for their low toxicity, mitigating environmental impacts (Silva et al., 2014).

The flotation can have its efficiency increased by adjusting operational parameters such as a pre-saturation of the effluent itself and with the use of biodegradable surfactants, which increase the adhesion of microbubbles

to oil droplets (Rocha e Silva et al., 2018). Campello Filho et al. (2020), for example, demonstrated an oil removal of 92.0% when using the crude and formulated biosurfactant produced by bacteria of the species *Pseudomonas cepacia* CCT6659 in a tower-induced saturation process. Thus, the aim of the present study was to evaluate the effect of using a biosurfactant produced by *Bacillus methylotrophicus* UCP1616 as a natural collector associated with an induced air pre-saturation chamber (IPSC), for the treatment of oily effluent.

2. Material and Methods

2.1 Obtainment of The Materials

A synthetic effluent with about 50 ppm of oil was used. The residual engine lubricating oil was obtained from an automotive maintenance establishment in the city of Recife, Pernambuco. The bacterium *Bacillus methylotrophicus* UCP1616 was obtained from the Bank of Cultures of the Nucleus for Research in Environmental Sciences and was used as a producer of biosurfactant.

Thus, the production of the biosurfactant was carried out in a mineral medium, because the literature has already described that microorganisms are capable of producing biosurfactants from various sources such as heavy mineral oils. The mineral medium was supplemented with 3% of cane molasses and 3% of millet. The fermentations for the production of the biosurfactant were carried out in Erlenmeyer flasks containing 500 mL of the medium with an inoculum at a concentration of 3% (v/v), for 48 hours at 28ºC at 200 rpm. The metabolic liquid containing the biosurfactant was centrifuged at 5000 rpm for 30 min in order to separate the microbial biomass, in order to obtain the crude biosurfactant, because the bacteria when grown in industrial waste have potential as a biosurfactant producer, according to Chaprão et al. (2018a).

Subsequently, the surface tensions were measured in the cell-free metabolic liquid in a KSV Sigma 700 tensiometer (Finland) using the NUOY ring. The determination of the Critical Micelle Concentration (CMC) of the biosurfactant corresponds to the minimum concentration of surfactant necessary for the surface tension of the water to be reduced to the maximum (Chaprão et al., 2018b). Then, the cell-free metabolic liquid containing the biosurfactant was subjected to the addition of 0.2% of potassium sorbate as a preservative, as described by Soares da Silva et al. (2019) that this preservative maintains its surfactant properties for a long time of storage in sterile conditions in a container.

Then, the cells were removed from the culture medium by centrifugation at 5000 rpm for 30 min to extract the biosurfactant. The HCl (6.0 M) was used to adjust the pH of the supernatant to 2.0, followed by the addition of an equal volume of $CHCl_3/CH_3OH$ (2:1). After vigorous stirring for 15 min, the mixture was put to rest until phase separation. After removing the organic phase, the procedure was repeated two more times. A rotary evaporator was used to concentrate the product from the organic phases. A yellowish viscous product was obtained which was dissolved in methanol and concentrated by evaporating the solvent at 45°C. The isolated biosurfactant was weighed (Durval et al., 2018).

2.2 Experimental Arrangement

The tests were performed in a flotation chamber with Induced pre-saturation on a bench scale, according to a schematic representation (Figure 1). A volume of 100 L of oily effluent added from the collector individually used in the process in the following volumes: crude biosurfactant from *B. methylotrophicus* (1.5 L), formulated biosurfactant from *B. methylotrophicus* (1.5 L), isolated biosurfactant from *B. methylotrophicus* at ½ x CMC (300 mg/L) and the commercial collectors of SIGMA-ALDRICH at ½ x CMC (150 mg/L) and sodium dodecyl sulfate (SDS) (1.2 mg/L), were homogenized for approximately 30 minutes to obtain a distribution uniform between water/oil/collector. The oily effluent without the addition of the collector was used as a control.

In the mentioned experimental arrangement, oily water is aspirated from the feed tank (1) by a centrifugal pump (2) adapted to previously saturate the effluent with atmospheric air to be treated. The air enters in the system quantified and regulated with the aid of a rotameter (3) and a needle-type valve (4). The saturation process takes place inside the pump and in the discharge line, under a pressure gauge of about 5.5 bar, maintained with the aid of a control valve (5). The influent, pre-saturated with air bubbles, enters the flotation chamber (6) where the oil-water separation occurs. It is essential to maintain the effluent level at the height of the chamber lid (7). This condition is required so that the oily foam formed and floated is pushed into the pipe connected to the highest part of the lid (8) that serves as the oily foam discharge pipe. The treated water gets out in the base of the flotation chamber, through a pipe that forms a hydraulic stamp (9), to the maintenance of the saturated effluent level in the chamber. At the base of the chamber of the pre-saturated effluent flotation, a valve was installed to remove samples from the treated water (10). At the top of the pipe that forms a stamp, it was connected a stretch of opened pipe towards the atmosphere (11), serving vacuum breaker when the

process stops to the maintenance or cleaning. A gate valve is closed during the operation of the system, serving to produce a vacuum by the Venturi effect, during the unblocking of the pipe of the oily foam discharge.

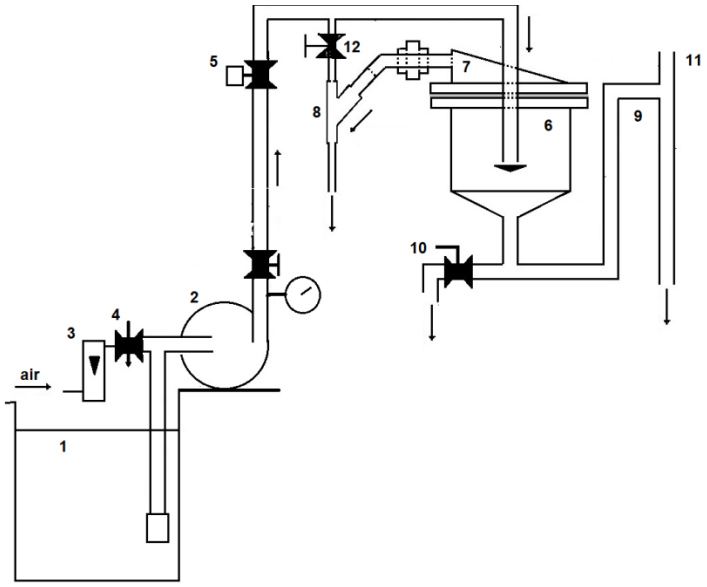

Figure 1: Flotation system with pre-saturation by air induced effluent on bench scale

The flotation system of the experimental arrangement in Figure 1 was built with a chamber of 3.4 L of effective volume and operated with a nominal flow of 2.0 $L \cdot min^{-1}$. Under these conditions, the hydraulic retention time was 2:50 min. The air flow for saturation of the synthetic effluent used was 2.0 $L \cdot min^{-1}$. Samples of the treated effluent were collected after 4, 8, 12, 16 and 20 minutes of the process to evaluate the percentage of oil removal.

The oil was extracted from the samples of the synthetic oily effluent, which was treated with the same volume of hexane (1:1, v/v). The mixture was vigorously stirred for 15 min and left to stand for phase separation. The organic phase was removed. After the extractions, the results were obtained using a UV-Vis spectrophotometer (SP-22-BIOSPECTRO) and the readings were performed at a wavelength of 330 nm in relation to a calibration curve prepared with a standard oil solution at 5000 mg/L in a 100 mL volumetric flask (Figure 2).

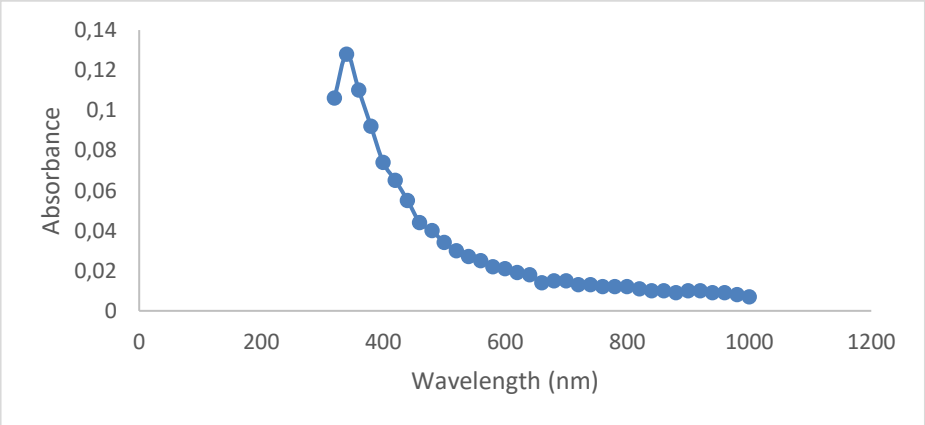

Figure 2: Obtaining the wavelength required to estimate residual oil concentrations

According to Emmandi et al. (2014), only the wavelength of 330 nm was used, as it approaches the ideal range for determining benzene using the hexane solvent with a determination coefficient of 0.9999. This wavelength is confirmed in the Figure 2. Benzene is one of the main components of the oil under study. The solutions were diluted in n-hexane in concentrations ranging from 1 to 1000 mg/L obtained from the initial

sample. N-hexane was used as the white for the device calibration. The solvent was analytical grade and appropriate for the spectrophotometric equipment. All experiments were performed in triplicate at room temperature (27°C) and average values were reported.

3. Results and Discussion

3.1 Evaluation of the Collectors in the Flotation Process in the Bench-induced Saturation Tower

The collectors used showed satisfactory removal results (Figure 2). However, it is important to note that the assay without the addition of a collector, with the action of microbubbles alone, showed descending percentages of removal over time, reaching 80.16% removal. Tests with the addition of biosurfactant of *Bacillus methylotrophicus* UCP1616, in the crude, formulated and isolated versions, showed ascending percentages of removal as the residence time of the collector increases in relation to the effluent, the crude biosurfactant reached 98.0% of removal, the formulated biosurfactant reached 98.39% removal and isolated biosurfactant reached 99.0% removal. The commercial biosurfactant reached 90.52% removal and the chemical surfactant SDS obtained 86.74% removal. The efficiency of oil removal in relation to the residence time of the biosurfactant under study should be further studied.

The results of the present study were superior to those reported by Campello Filho et al. (2020), which in turn demonstrated an oil removal of 97.49% with isolated biosurfactant of *Pseudomonas cepacia* CCT6659 in an induced saturation tower. Chaprão et al. (2018b) also used biosurfactant of *Bacillus methylotrophicus* UCP1616, in a DAF, with a 92.0% oil removal rate. The biosurfactant of *Candida sphaerica* UCP 0995 studied by Rocha e Silva et al. (2015) added considerable value to the DAF process, increasing from 80.0% to 98.0% the efficiency of removing oil derivatives in the dissolved air flotation (DAF) prototype.

The results obtained in this study revealed advantages in the use of the IPSC over the mentioned horizontal DAF system. The IPSC system obtained a 7.0% increase in oil removal efficiency compared to horizontal DAF, using the same biosurfactant produced by *B. methylotrophicus*. The IPSC had a greater efficiency in removing the flow-microbubble complex in relation to the traditional DAF by having air injection in favour of the flow (concurrent flow), whereas in traditional DAF systems an orthogonal air flow in relation to the horizontal of effluent.

The same direction of the flow of microbubbles in relation to the flow of the effluent allows for a longer contact time between the microbubbles and the oil and this explains the greater removal efficiency in the IPSC process than in the DAF process whose flow of microbubbles is perpendicular to effluent flow.

It is observed in Figures 3, a relevant increase in the efficiency of the tests carried out in the time of 20 minutes, promoted by the longer residence time of the effluent, thus obtaining a greater contact of the injected air in favour of the flow with the effluent in the float and consequently obtain an increase in efficiency. The increase in oil removal occurred linearly, because it occurred gradually.

Surfactants allow oil droplets to adhere to microbubbles, which in turn have different polarities, which explains the greater separation efficiency in processes with surfactants. The greater removal efficiency in the process with biosurfactant is explained by its greater stability than in the process with SDS. The difference in oil removal between rhamnolipids and the biosurfactant *Bacillus methylotrophicus* occurs due to the high specificity that the class of biosurfactants has. The formulated biosurfactant was superior to the crude biosurfactant due to the greater stability promoted by potassium sorbate and the best result was found with the isolated biosurfactant due to the higher concentration of the tensoactive compound.

The biosurfactant has removal efficiency with increased residence time; but if there is a big increase in residence time, the process is economically unfeasible. The residence time depends on the level of the contaminants contained in the effluent to be treated, a larger amount of contaminant would require a longer residence time.

It was perceived in this treatment that with a low affluent flow and a high biosurfactant flow the flotation process was optimized. The best operation occurred with an affluent flow of 4.00 L/min and a flow of 0.60 L/min of the surfactant responsible for the formation of foams in the system. Surfactants are amphipathic molecules, and when attached to microbubbles and drops of oil further reduces the density of the hydrophobic part of the flotation in relation to water, facilitating the separation with the formation of foams. In this case, the biosurfactant acts to break emulsions, but, in other cases, biotensoatives can form emulsions.

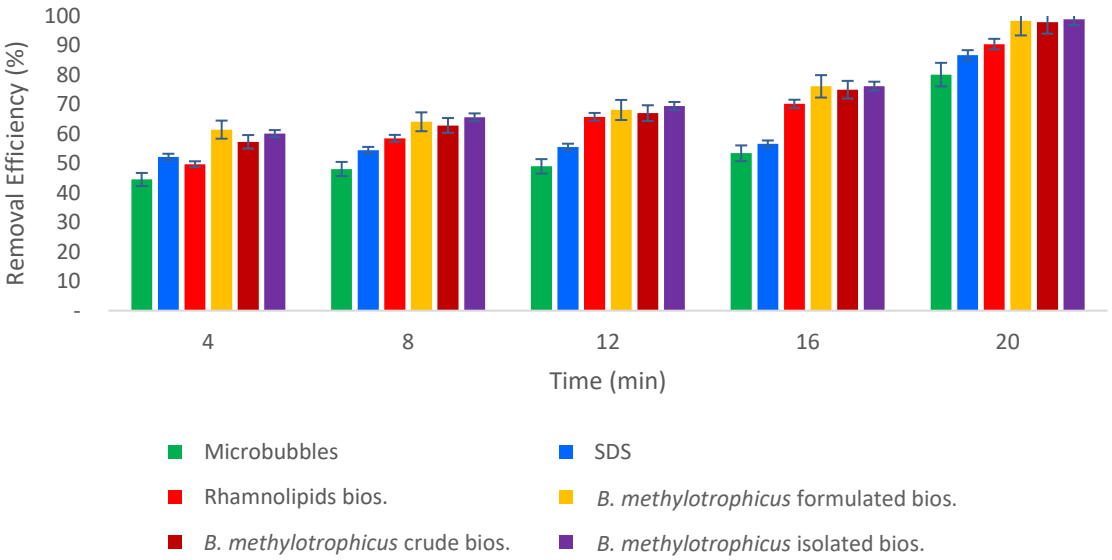

Figure 3: Results of oil removal by the action of surfactants in the flotation process with IPSC on a bench scale

3.2 Statistical Analysis

Analysis of Variance (ANOVA) was used to verify statistically significant differences between the mean values of the responses induced by variations in the independent variables. For this purpose, after preliminary comparison between the data that composed each midpoint with the help of Box plot, the one-way ANOVA test was used. The differences were considered statistically significant when the level of significance (p) was <0.0001.The model diagnosis was realised through the ANOVA, where the values approximate the normal line and, therefore, the assumption of data normality is met.
Based on the graph of Box plot type, it was possible to evaluate the distribution of experimental data from the efficiency tests on the float. This type of graph lets us observe how the essays are distributed. Furthermore, we can obtain, in this graph, values of central tendency (median), maximum and minimum values and, if any, outliers (Figure 4).

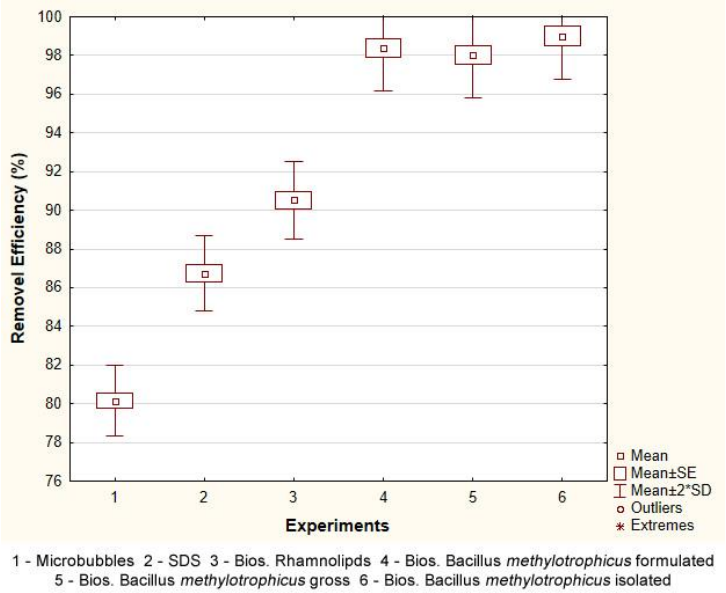

Figure 4: Flotation Test Box Diagram

The box plot graphs (Figure 4) demonstrated that there were no significant errors due to the absence of Outliers and Extremes. The low difference between the upper and lower limits of each treatment, as shown in the graphs, refers to a high precision of the data. Through these graphs it is possible to observe that the arithmetic means are close to the medians, that is, a normal distribution is present. Finally, the box plot

graphics make it evident that the treatment with the biosurfactant isolated from *Bacillus methylotropicus* showed an oil removal efficiency superior to the other treatments.

4. Conclusions

The *Bacillus methylotrophicus* biosurfactant showed promising results with 99.00% oil removed compared to commercial collectors during the comparative study. The results obtained in this work proved the great potential of the biosurfactant under study to be used as an auxiliary collector in the treatment of oily effluents in the flotation system configured in a chamber with pre-saturation by air induced on a bench scale. The *B. methylotrophicus* biosurfactant applied to flotation led to a significant increase in the oil removal rate, making the process of treating oily effluents more suitable for an industrial environment. The study is important for the design of a float at an industrial level in the future. The biosurfactant makes it is possible to use a smaller tank and obtain a shorter oil removal time, making the process economically viable.

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