

Research Article

Bioconversion of Agroindustrial Waste in the Production of Bioemulsifier by *Stenotrophomonas maltophilia* UCP 1601 and Application in Bioremediation Process

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This study investigated the potential of the bacterium *Stenotrophomonas maltophilia* UCP 1601 to produce a new biomolecule with emulsifying properties by determining the hemolytic activity, obtaining a halo of 9 mm in blood agar. Fermentations were carried out in saline mineral medium supplemented with 10% waste soybean oil (WSO) and different concentrations of glucose, peptone, ZnCl₂, and MgSO₄, according to a 2⁴ full-factorial design. The results showed that the best results were obtained in condition 6 (medium composed of 4% glucose, 1% peptone, 2.72% ZnCl₂, and 2.46% MgSO₄), with excellent high emulsification index of 82.74%, using burned motor oil. The emulsifying property of the biomolecule produced was confirmed by the emulsification index of 78.57, 54.07, and 58.62%, using soybean, corn, and diesel oils, respectively, and the stability at different values of pH, temperature, and NaCl concentrations. The yield of the produced bioemulsifier was 2.8 g/L, presenting an anionic character and polymeric nature (37.6% lipids, 28.2% proteins, and 14.7% carbohydrates), confirmed by FTIR. The new bioemulsifier demonstrated promising potential for bioremediation of hydrophobic contaminants in the environment, since it had the ability to reduce the viscosity of WSO and burned motor oil, as well as excellent dispersion capacity of the burned motor oil in water (69.94 cm² of oil displacement area), and removing 71.7% of this petroleum derivative from sandy soil.

1. Introduction

Bioemulsifiers are amphipathic molecules with hydrophobic and hydrophilic moieties that act between fluids of different polarities (oil/water and water/oil), allowing access to hydrophobic substrates and increasing the hydrocarbon contact area, mobility, bioavailability, and biodegradation of such compounds [1–4]. They have a tendency to change the physical and chemical properties of the fluids, causing the ability of detergency, lubrication,

foaming and solubilization, emulsification, and reduction in liquid viscosity [5, 6].

Bioemulsifiers are produced by a wide variety of microorganisms, including bacteria, yeast, and filamentous fungi. When compared to synthetic sources, microbial emulsifiers have several advantages such as stability to wide ranges of pH, temperature and salinity, biodegradability, and low toxicity, which make them promising in many industrial applications [7]. Bioemulsifiers have been widely used in advanced oil recovery, bioremediation of hydrocarbon

polluted environments, removal of heavy metals and chlorinated solvent compounds, etc [8, 9].

Due to population growth and recent patterns of consumption, there was an increase in the production of agroindustrial and agricultural systems. A large amount of wastes is generated because of the unproductive increase in productivity, leading to economic, social, and environmental damage [10]. The use of residues as alternative substrates for formulation of culture media to produce bioemulsifiers provides carbohydrate and/or lipid levels required to support microbial growth and bioemulsifier biosynthesis, favoring microorganism growth and metabolite production [11–13].

Stenotrophomonas maltophilia is a Gram-negative bacterium that inhabits plants, soil, and water. Because of beneficial interactions with plants promoting growth, it has become important for biotechnological applications in agriculture [14, 15]. In addition, there are several studies in the literature on the biotechnological potential of *S. maltophilia* due to the production of enzymes such as lipases and proteases [16, 17]. Recently, some researchers have highlighted the potential to produce biosurfactants/bioemulsifiers, with effective larvicidal and/or pesticidal properties, effective in the degradation of diesel and used motor oil or in the solubilization of phenanthrene [18–20]. In this context, this study aimed the production of bioemulsifier by *S. maltophilia* UCP 1601, using waste soybean oil (WSO) as low-cost substrate, through a 2^4 full-factorial design (FFD). The isolation and preliminary characterization of bioemulsifier were carried out, as well as studies of its stability, effect in the viscosity of hydrophobic compounds, and effectiveness as a dispersing agent and in the removal of burned motor oil.

2. Materials and Methods

2.1. Microorganism. The microorganism used in this study was *Stenotrophomonas maltophilia* UCP 1601, isolated from clay soil on the Capibaribe River, Pernambuco, and identified by Vilar Júnior (date not published). The bacterium was deposited in Culture Collection of the Catholic University of Pernambuco, registered at the World Federation for Culture Collection (WFCC), and maintained in nutrient agar medium and 30% glycerol, at 5°C.

2.2. Materials. WSO and commercial detergent used in this study were obtained from informal commerce at the city of Recife, Brazil. Glucose, peptone, $ZnCl_2$, $MgSO_4$, and Tween 80 chemicals were purchased from Merck (Darmstadt, Germany). The chemical composition of WSO used in this study was reported by Andrade et al. [21].

2.3. Hemolysis Test. The potential of *S. maltophilia* UCP1601 in the production of bioemulsifier was investigated using the hemolytic activity test [22]. The bacterium was inoculated into the central part of the agar plate containing 5% (v/v) defibrinated sheep blood and incubated at 37°C for 24 h. The

activity was evaluated by the appearance of a clear zone around the *S. maltophilia* colony.

2.4. Production of Bioemulsifier by *S. maltophilia*

2.4.1. Preparation of the Inoculum. A colony of *S. maltophilia* UCP 1601 grown on nutrient agar was transferred to 50 mL of nutrient broth and incubated at 37°C and 150 rpm on an orbital shaker, for 24 h. Thereafter, the optical density of the culture medium was checked at 600 nm on a UV/Vis spectrophotometer Libra S32 (Biochrom Ltd.). Culture with optical density 0.8–1.0 at 600 nm was used as inoculum.

2.4.2. Production of Bioemulsifier. Bioemulsifier production was carried out in Erlenmeyer flasks containing 100 ml of saline mineral medium (MMS) (composition in g/L: 1.0 $(NH_4)_2SO_4$, 2.0 K_2HPO_4 , 0.5 KH_2PO_4 , 10.0 $MgSO_4 \cdot 7H_2O$, 5.0 NaCl, 0.2 $FeSO_4$, and 0.5 $CaCl_2$) [23] supplemented with 10% WSO. The medium was supplemented with glucose, peptone, $ZnCl_2$, and $MgSO_4$, according to a 2^4 FFD (Section 2.4.3). Production media were adjusted to pH 7 and sterilized by autoclaving for 15 min at 121°C. Then, they were inoculated at 10% and incubated at 30°C under orbital rotation (150 rpm) for 96 h. After this time, the media were subjected to centrifugation at 10000 rpm and 10°C for 15 min. The cell-free metabolic liquids obtained were used to evaluate the bioemulsifier production, by determining the emulsification index, as described later (Section 2.4.4).

2.4.3. Full-Factorial Design (FFD). In this study, a 2^4 FFD was used to evaluate the influence of four variables (glucose, peptone, $ZnCl_2$, and $MgSO_4$). Each independent variable was investigated at minimum (–) and maximum (+) levels, as shown in Table 1. Sixteen experimental assays were performed in triplicate, and the emulsification index was used as variable response. The experimental data were analyzed by Statistica® software, version 10.0 (StatSoft Inc., USA), and the significance of the results was tested ($p < 0.05$).

2.4.4. Determination of Emulsification Index (EI_{24}). The emulsification index (EI_{24}) was determined according to the methodology described by Cooper and Goldenberg [24]. In brief, 1.0 ml of cell-free metabolic liquid from each condition of FFD and 1.0 ml of burned motor oil were mixed in a test tube and vortexed thoroughly for 2 min at room temperature (25°C). The mixtures were kept at rest for 24 h, and after that, the index was determined by the following equation:

$$EI_{24} (\%) = \frac{H_e}{H_t} \times 100, \quad (1)$$

where H_e = height of the emulsion and H_t = total height of the mixture. The conditions of FFD with higher value of EI_{24} using burned motor oil were selected for determination of EI_{24} using soybean, corn, and diesel oil, by methodology described by Cooper and Goldenberg [24]. The results were

TABLE 1: Variables and levels used in the 2^4 FFD for the production of bioemulsifier by *Stenotrophomonas maltophilia* UCP 1601.

Variables	Levels	
	Low (-1)	High (+1)
Glucose (% w/v)	1	2
Peptone (% w/v)	0.5	1
ZnCl ₂ (mM)	50	100
MgSO ₄ (mM)	50	100

compared, and the condition with better EI₂₄ for all hydrophobic compounds was used for further studies.

2.5. Determination of the Stability of the Bioemulsifier. The stability of the bioemulsifier produced by *S. maltophilia* UCP 1601 in the selected condition of the FFD was determined through the EI₂₄. Cell-free metabolic liquid was submitted separately at different temperatures (0, 5, 30, 37, 50, and 100°C), adjusted to different pH values (2, 4, 6, 8, 10, and 12) or NaCl (2, 4, 6, 8, 10, and 12%). Then, EI₂₄ was determined using burned motor oil as a hydrophobic substrate following the methodology previously described in Section 2.4.4 [25, 26].

2.6. Effect of the Bioemulsifier on the Viscosity of Hydrophobic Compounds. The effect of the bioemulsifier produced by *S. maltophilia* UCP 1601 on the viscosity of hydrophobic compounds (WSO and burned motor oil) was investigated using the methodology described by Andrade Silva et al. [27] and Maia et al. [26]. The viscosity of the hydrophobic compounds was determined in test tubes before and after adding 2 ml of the cell-free metabolic liquid (crude bioemulsifier) of the selected condition of the FFD, and the mixture was vigorously homogenized in vortex for 1 min. The determination was made using an automatic viscometer (Brookfield (Middleboro, MA, USA) TC 500), and the results were expressed in centipoise (cP).

2.7. Oil Dispersion Test. To determine the potential as dispersing agent of the bioemulsifier produced by *S. maltophilia* UCP 1601, the oil dispersion test was performed by placing 40 mL of distilled water in a Petri dish (10 cm in diameter), followed by the addition of 1.0 mL of burned motor oil on the surface of the water layer. Then, 0.5 mL of cell-free metabolic liquid (crude bioemulsifier), commercial detergent, Tween 80, or distilled water was placed in the center of the oil film [27, 28]. The dispersion capacity of the bioemulsifier was evidenced by dispersion of the oil, resulting in the formation of oil displacement area (ODA). The assays were performed in triplicate; the clear zone diameters were measured and the respective ODA were determined and expressed in cm², using the equation below [27]:

$$\text{ODA} = 3.14 \times r^2. \quad (2)$$

2.8. Bioemulsifier Isolation. The bioemulsifier produced by *S. maltophilia* UCP 1601 was extracted from the supernatant using three methodologies of precipitation with organic solvents (acetone and ethanol 70%) (1 : 1, v/v). The mixtures were allowed to stand for 24 h at 5°C, and then the precipitate was obtained by centrifugation at 5000g for 15 min at 5°C. The supernatants were discarded, and the precipitate was washed twice with distilled water and dried at 70°C for 3 h. Bioemulsifier yields were expressed as g/L and compared in order to select the more efficient method for bioemulsifier isolation [27].

2.9. Characterization of Bioemulsifier. Protein concentrations in the bioemulsifier were determined using the Labtest kit (Labtest Diagnostica S.A., Minas Gerais, Brazil) for identification of the total protein content. The total carbohydrate content was estimated using the phenol-sulfuric acid method [29]. The lipid content was determined according to Manocha et al. [30].

Fourier transform infrared spectroscopy (FTIR) was carried out on a spectrometer (BRUKER, Karlsruhe, Germany) by FTIR-attenuated total reflection (ATR) technique. The isolated bioemulsifier was analyzed by measuring in the range of 4000–600 cm⁻¹.

The ionic charge of the bioemulsifier was determined using a Zeta potentiometer model ZM3-D-G, Zeta Meter System 3.0+, with direct images for Zeta Meter video, San Francisco, CA, USA [31].

2.10. Application of Bioemulsifier in Removal of Burned Motor Oil from Contaminated Sand. The suitability of the bioemulsifier for removing burned motor oil was investigated using the methodology of Luna et al. [32]. Twenty-gram samples of sand were transferred to 250 mL Erlenmeyer flasks, artificially contaminated with 20 mL of burned motor oil and submitted to the following treatments: (A) addition of 150 mL of distilled water (control), (B) addition of 150 mL of 0.5% SDS solution, and (C) addition of 150 mL of cell-free metabolic liquid (crude bioemulsifier). The flasks were subjected to 150 rpm for 48 h at 28°C and then centrifuged at 5000 g for 20 min for separation of the washing solution and sand sediment. The amount of oil remaining in the sand was gravimetrically determined by hexane.

3. Results and Discussion

3.1. Hemolytic Activity of *S. maltophilia* UCP 1601. Several researchers have studied hemolytic activity as a criterion for the selection of microorganisms producing surfactants [6, 22, 33]. The microorganisms with positive hemolytic activity show a clear zone in the blood agar plates, which was confirmed for *S. maltophilia* UCP 1601. During its radial growth in the medium, the bacterium formed a halo of 9 mm in diameter, after 24 h of incubation. Previously, Hemlata et al. [23] reported the formation of a halo of 13 mm by *S. maltophilia* NBS-11. Similarly, other researchers have confirmed the positive hemolytic activity of

biosurfactant-producing *Stenotrophomonas maltophilia* strains [18, 34].

3.2. Production of Bioemulsifier by *S. maltophilia* UCP 1601.

Determination of emulsification index has often been used as a suitable method for identifying emulsifiers which are markedly characterized by their excellent ability of emulsion stabilizing [4, 25, 33].

Table 2 presents the results of emulsification index of the cell-free metabolic liquid, obtained by *S. maltophilia* UCP 1601 after culture in the 16 conditions of the FFD. The values of emulsification index obtained experimentally and predicted by the statistical model used to analyze the 2^4 FFD are shown in the Supplementary Material (Table 3).

The property of emulsion stabilization can be evaluated by the ability to maintain at least 50% of the original emulsion volume after 24 hours of formation [35]. Thus, the biomolecule produced by *S. maltophilia* UCP 1601 presents emulsifying property with burned motor oil, with EI_{24} of 81.97% and 82.74%, in condition 4 and 6 of FFD, respectively. However, when tested with other hydrophobic compounds, the emulsification index was less than 50% to bioemulsifier of condition 4, whereas that obtained in condition 6 showed EI_{24} of 78.57, 54.07, and 58.62%, with soybean, corn, and diesel oils, respectively (Figure 1). Thus, condition 6 was selected for the following studies.

3.3. Statistical Analysis. In this study, a 2^4 FFD was used to analyze the influence of 4 components of the culture medium (glucose, peptone, $ZnCl_2$, and $MgSO_4$) on the production of bioemulsifiers by *S. maltophilia* UCP 1601. The response or dependent variable used was the emulsification index and the following equation was used to establish its relation with the independent variables:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + b_{123}x_1x_2x_3 + b_{124}x_1x_2x_4 + b_{134}x_1x_3x_4 + b_{234}x_2x_3x_4, \quad (3)$$

where Y is the response variable (emulsification index); b_0 is a constant; b_1 , b_2 , b_3 , and b_4 are the regression coefficients for the linear; and b_{12} , b_{13} , b_{14} , b_{23} , b_{24} , b_{34} , b_{123} , b_{124} , b_{134} , and b_{234} are the interaction coefficients.

In order to analyze the mathematical model, adjustments were made by the linear regression methods, and Table 3 (Supplementary material) shows the values obtained experimentally and those predicted by the model, to obtain the highest value of the emulsification index. Figure 2 of Supplementary Material represents the relation between these values, showing their distribution near the line, which indicates that equation (4) proposed by the mathematical model explains the values of emulsification index obtained. This was evidenced by the high coefficient of determination ($R^2 = 0.9993$) obtained in the analysis of variance (ANOVA) represented in Table 4 of the Supplementary Material. Reproducibility of the experimental data was confirmed by the

TABLE 2: Full-factorial design used for the production of bioemulsifier by *S. maltophilia* UCP 1601 after 96h of culture in medium containing 10% WSO. The tests were performed in triplicate and the media of emulsification index (EI) values were used as response variable.

Assays	Glucose	Peptone	$ZnCl_2$	$MgSO_4$	EI_{24} (%)
1	-1	-1	-1	-1	53.84
2	+1	-1	-1	-1	60.71
3	-1	+1	-1	-1	61.90
4	+1	+1	-1	-1	81.97
5	-1	-1	+1	-1	68.00
6	+1	-1	+1	-1	82.74
7	-1	+1	+1	-1	76.42
8	+1	+1	+1	-1	40.62
9	-1	-1	-1	+1	72.41
10	+1	-1	-1	+1	59.09
11	-1	+1	-1	+1	63.81
12	+1	+1	-1	+1	78.94
13	-1	-1	+1	+1	42.85
14	+1	-1	+1	+1	79.44
15	-1	+1	+1	+1	59.09
16	+1	+1	+1	+1	65.62

Glucose (% w/v) level -1 (1) and +1 (2); Peptone (% w/v) level -1 (0.5) and +1 (1.0); $ZnCl_2$ (mM) level -1 (50) and +1 (100); and $MgSO_4$ (mM) level -1 (50) and +1 (100).

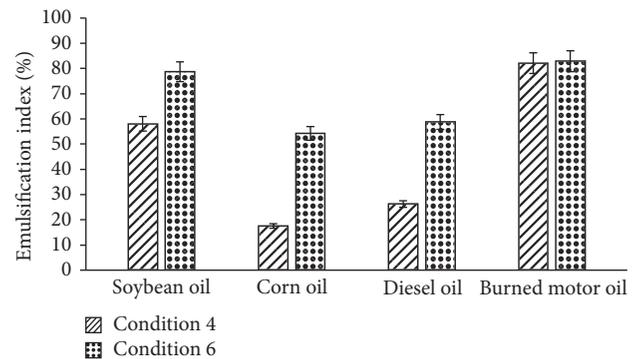


FIGURE 1: Emulsification index of bioemulsifier produced by *Stenotrophomonas maltophilia* UCP 1601 in conditions 4 and 6 of the 2^4 FFD, using different hydrophobic substrates.

value of the adjusted determination coefficient (adjusted $R^2 = 0.9898$), showing also that the mathematical model described is in agreement with the experimental data.

$$Y = 65.46562 + 3.17562x_1 + 0.58062x_2 - 1.11813x_3 - 0.30937x_4 - 2.43438x_1x_2 - 0.41813x_1x_3 + 2.44063x_1x_4 - 4.49062x_2x_3 + 1.12813x_2x_4 - 2.28812x_3x_4 - 7.64062x_1x_2x_3 + 2.23312x_1x_2x_4 + 5.58188x_1x_3x_4 + 3.38688x_2x_3x_4. \quad (4)$$

The ANOVA also indicated that for the experimental data obtained, only the interactions of glucose, peptone, and $ZnCl_2$ ($1 * 2 * 3$); glucose, $ZnCl_2$, and $MgSO_4$ ($1 * 3 * 4$); and peptone and $ZnCl_2$ ($2 * 3$) were statistically significant on the

emulsification index when analyzed at the 95% confidence level. Only the interaction of glucose, ZnCl_2 , and MgSO_4 ($1 * 3 * 4$) showed a positive effect on the emulsification index, considering the influence of the independent variables (glucose, peptone, ZnCl_2 , and MgSO_4) on the dependent variable (emulsification index) as shown in Table 4 (Supplementary material).

3.4. Stability of the Bioemulsifier. The use of microbial emulsifiers in various industrial areas depends on their stability under different temperatures, pH, and salinity conditions [36, 37].

The results showed that the emulsification index remained practically unchanged (above 80%) in relation to the different values of pH and salinity tested, with only a small decrease to 2% of NaCl (Figures 2(a)–2(c)). However, in the case of temperature, the extreme values (0 and 100°C) affected the emulsification (Figure 2(b)), which is probably caused by partial precipitation of the bioemulsifier [38, 39]. Similar results were reported by Gargouri et al. [19] when they evaluated the stability of the biosurfactant produced by *Stenotrophomonas* sp.

3.5. Effect of the Bioemulsifier on Viscosity of Hydrophobic Compounds. In this study, the effect of the bioemulsifier produced by *S. maltophilia* UCP 1601 on the viscosity of soybean oil and burned motor oil was evaluated. The results showed a decrease in viscosity of both compounds tested, from 380.1 to 21.6 cP, for soybean oil, and from 148.9 to 46.9 cP for the burned motor oil. Similarly, the biosurfactant produced by *Candida glabrata* UCP 1556 decreases the viscosity of soybean oil to 18.5 cP [35], and the authors have suggested its application in the cosmetic formulation.

Recently, Maia et al. [26] reported a decrease in the viscosity of burned motor oil by the bioemulsifier produced by *Bacillus subtilis* UCP 0146. This property to viscosity reduction is desirable in emulsifiers with potential application in the oil industry as it contributes to improved oil recovery, heavy oils, or cleaning contaminated sites [26, 40].

3.6. Dispersing Properties of the Bioemulsifier Produced by *S. maltophilia*. Chemical dispersants are widely used in various industrial applications, such as paints and coatings, oil and gas, construction, pharmaceuticals, paper and cellulose, agricultural products, detergents, and others [41]. However, its constant use can cause high environmental impact due to the toxicity and non-biodegradability of some components of its formulation [42, 43]. Therefore, there is a growing demand for environmentally friendly and low-cost dispersants and, in this context, microbial surfactants were considered promising candidates [44, 45].

In this study, the dispersion capacity of the bioemulsifier produced by *S. maltophilia* UCP 1601 was investigated using the oil dispersion test using burned motor oil. As shown in Figure 3, the bioemulsifier showed excellent dispersion potential with 69.94 cm² of ODA, similar to Tween 80

(73.38 cm²) and higher than that of commercial detergent (24.49 cm²).

The results obtained by the bioemulsifier produced by *S. maltophilia* UCP 1601 were superior to those of other strains of this species [20, 23]. Recently, Gargouri et al. [19] reported the formation of a 6 cm diameter oil dispersion halo by the biosurfactant produced by *Stenotrophomonas* sp. B-2, which corresponds to an ODA of 28.27 cm². In addition, emulsifiers produced by bacteria of other genera obtained lower values of ODA, as reported by Maia et al. [26] for the bioemulsifier produced by *Bacillus subtilis* UCP 0146 (55.38 cm²). Thus, the bioemulsifier produced in this study shows its potential application as a dispersing agent in oil spills or industrial formulations.

3.7. Bioemulsifier Isolation and Yield. For many biotechnological products, the downstream processing costs account for 70%–80% of the total production costs [5]. Several conventional methods for the recovery of bioemulsifiers, such as acid precipitation, solvent extraction, ammonium sulfate precipitation, and centrifugation and foam fractionation have been widely reported [46, 47]. In this study, three methods of recovery were tested (Section 2.8). Ethanol extraction obtained better bioemulsifier yield (2.8 g/L) followed by acetone (1.7 g/L). Little amount of the bioemulsifier was recovered by acid precipitation (0.9 g/L). Then, extraction with ethanol was considered the most effective method for biomolecule isolation.

3.8. Characterization of Bioemulsifier. The zeta potential determines the ionic charge of the particle, which serves to predict and control the stability of colloidal suspensions and emulsions. Higher values of zeta potential indicate good stability of the suspension, due to the repulsion between hydrophilic particles, according to the literature [22, 27]. According to the analyses using a Zeta Potential Meta 3.0+, the bioemulsifier produced by *S. maltophilia* showed an anionic character (-33.97 ± 0.23 ZPmv, at 28.3°C).

Preliminary biochemical characterization showed that the bioemulsifier had a polymeric structure, composed of lipids (37.6%), proteins (28.2%), and carbohydrates (14.7%). Similarly, Araújo et al. [48] carried out these methodologies to obtaining the biochemical composition of biosurfactant produced by *Serratia marcescens* UCP 1549 and revealed the presence of 43% lipids, 32% proteins, and 11% carbohydrates, suggesting its polymeric nature. The biosurfactant obtained by *Stenotrophomonas* sp. B-2 was structurally characterized, containing cyclic peptides and lipid structures in nature, whereas the purified biosurfactant of *S. maltophilia* NBS-11 contained 34% carbohydrates, 62% lipids, and 4% proteins.

FTIR spectrum displays a broad peak at 3312.13 cm⁻¹ elucidating OH group (Figure 4). The stretch 1652.30 cm⁻¹, –C [double bond, length as m-dash] O stretch, and carboxyl –C–O stretch formed (2925.11). Additionally, two stretch signals appeared at 1018.70 cm⁻¹ where the presence of C=O in the glycolipid structure was confirmed, according to Jadhav et al [49]. The region between 672.37 cm and 1 is

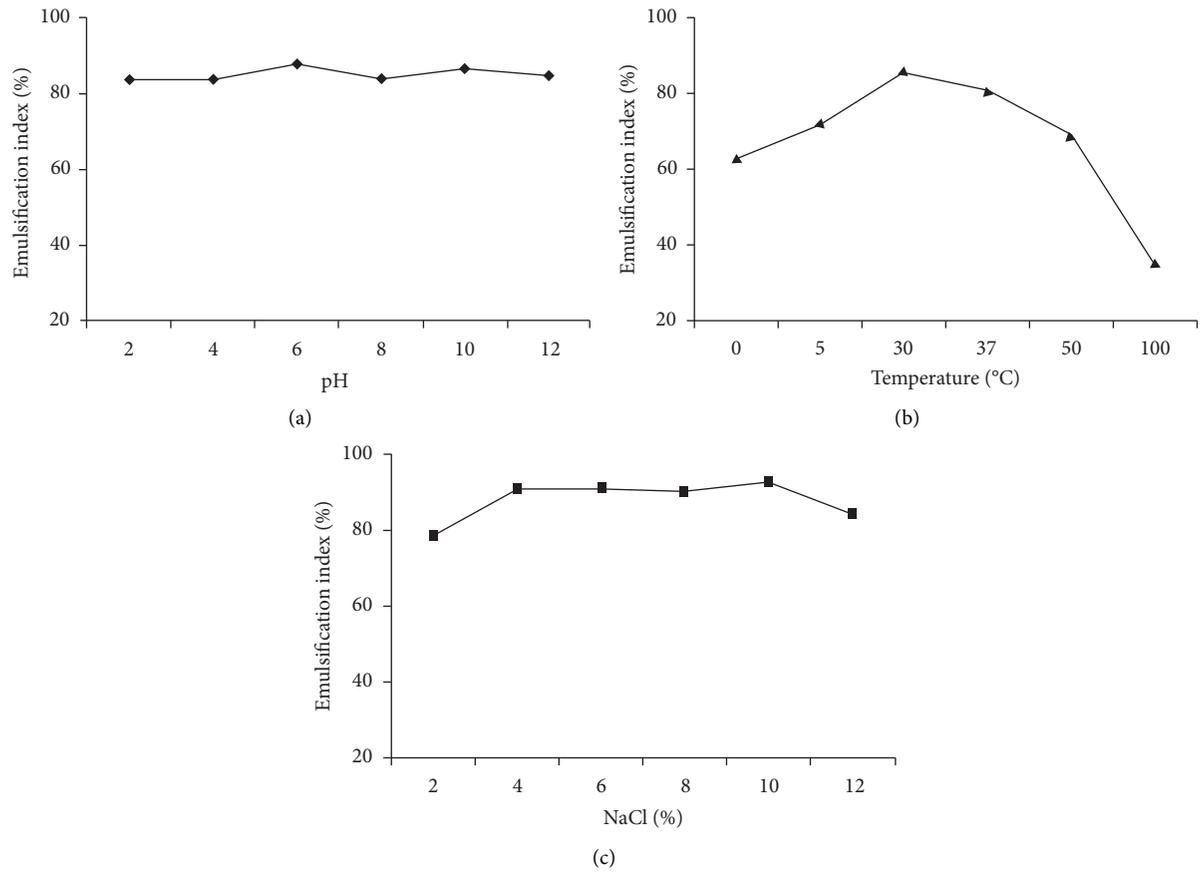


FIGURE 2: Stability of the bioemulsifier produced by *S. maltophilia* UCP 1601 against different values of pH (a), temperature (b), and small decrease in salinity concentrations (c).

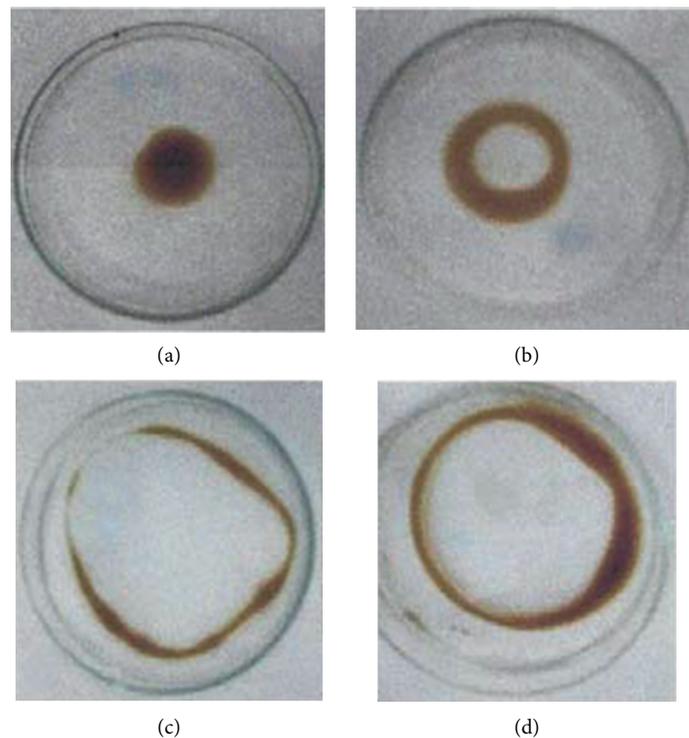


FIGURE 3: Dispersion area of the burned motor oil according to the compound used: distilled water (a), commercial detergent (b), Tween 80 (c), and crude bioemulsifier produced by *Stenotrophomonas maltophilia* UCP 1601 (d).

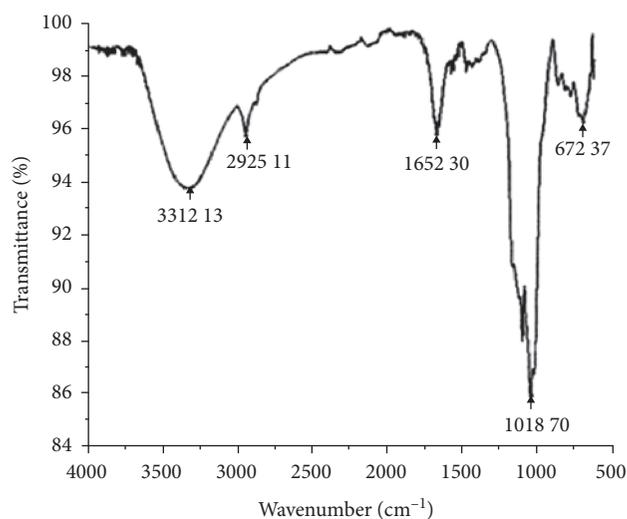


FIGURE 4: FTIR analysis provides specific information about chemical structure of the bioemulsifier produced by *Stenotrophomonas maltophilia* UCP1601.

considered a carbohydrate fingerprint and often presents some peaks related to anomeric carbon [50]. The results indicate the obtained bioemulsifier is mainly a complex of lipopeptides with small fraction of glycolipids suggesting a polymeric molecule.

3.9. Application of Bioemulsifier in Removal of Burned Motor Oil from Contaminated Sand. Bioemulsifiers can emulsify hydrocarbons enhancing their water solubility and increasing the displacement of oil substances from soil particles [40]. In this study, promising results were obtained by the crude bioemulsifier of *S. maltophilia* with removal of 71.64% of burned motor oil adsorbed on sandy soil samples, when compared with SDS (81.47%) and distilled water (6.2%) as controls. Therefore, it was evidenced the considerable potential of crude bioemulsifier for use in oil bioremediation processes, as alternative to synthetic counterpart.

4. Conclusions

S. maltophilia UCP 1601 has demonstrated its potential in the production of anionic and polymeric bioemulsifier with excellent dispersing properties, as well as effective in reducing the viscosity of hydrophobic compounds and removal of burned motor oil from sand. Thus, it was demonstrated its biotechnological potential in the bioremediation of contaminated ecosystems with hydrophobic pollutants derivative molecules from petroleum.

Data Availability

The data on factorial design and submerge fermentation obtained experimentally during the accomplishment of the Master Course in “Environmental Processes Development of Catholic University of Pernambuco-UNICAP” are included within the article. The emulsification index (%) obtained

experimentally and predicted from bioemulsifier produced by *Stenotrophomonas maltophilia* are included within the supplementary information files.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Materials

Figure 2: predicted values versus observed values by model for the response variable (emulsification index) from the results obtained in the 2^4 FFD. Table 3: values of emulsification index obtained experimentally and predicted by the statistical model used to analyze the 2^4 FFD. Table 4: ANOVA obtained from the results obtained in the 2^4 FFD to analyze the influence of the independent variables (glucose, peptone, $ZnCl_2$, and $MgSO_4$) on the dependent variable (emulsification index). (Supplementary Materials)

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