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Application of Green Surfactants in the Remediation of Soils Contaminated by Hydrocarbons

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Abstract: Among the innovative technologies utilized for the treatment of contaminated soils, the use of green surfactants appears to be a biocompatible, efficient, and attractive alternative, since the cleaning processes that normally use synthetic surfactants as additives cause other problems due to toxicity and the accumulation of by-products. Three green surfactants, i.e., two biobased (biobased 1 and biobased 2) surfactants produced by chemical synthesis and a microbial surfactant produced from the yeast *Starmerella bombicola* ATCC 22214, were used as soil remediation agents and compared to a synthetic surfactant (Tween 80). The three surfactants were tested for their ability to emulsify, disperse, and remove different hydrophobic contaminants. The biosurfactant, which was able to reduce the water surface tension to 32.30 mN/m at a critical micelle concentration of 0.65 g/L, was then used to prepare a commercial formulation that showed lower toxicity to the tested environmental bioindicators and lower dispersion capacity than the biobased surfactants. All the green surfactants showed great emulsification capacity, especially against motor oil and petroleum. Therefore, their potential to remove motor oil adsorbed on different types of soils (sandy, silty, and clay soil and beach sand) was investigated either in kinetic (flasks) or static (packed columns) experiments. The commercial biosurfactant formulation showed excellent effectiveness in removing motor oil, especially from contaminated sandy soil (80.0 ± 0.46%) and beach sand (65.0 ± 0.14%) under static conditions, while, in the kinetic experiments, the commercial biosurfactant and the biobased 2 surfactant were able to remove motor oil from all the contaminated soils tested more effectively than the biobased 1 surfactant. Finally, the *S. bombicola* commercial biosurfactant was evaluated as a soil bioremediation agent. In degradation experiments carried out on motor oil-contaminated soils enriched with sugarcane molasses, oil degradation yield in the sandy soil reached almost 90% after 60 days in the presence of the commercial biosurfactant, while it did not exceed 20% in the presence of only *S. bombicola* cells. These results promise to contribute to the development of green technologies for the treatment of hydrophobic pollutants with economic gains for the oil industries.

Keywords: green surfactant; biobased surfactant; biosurfactant; petroleum; bioremediation; biodegradation; *Starmerella bombicola*

1. Introduction

The contamination of soils, oceans, and seas has contributed to increased research on environmental remediation. Petroleum hydrocarbons, heavy metals, and agricultural pesticides have mutagenic, carcinogenic, immunotoxic, and teratogenic effects and cause drastic changes in soil physicochemical and microbiological characteristics, thereby representing a serious threat to health and the environment. Therefore, soil pollution urgently requires the application of a series of physicochemical and biological techniques and treatments to minimize the extent of damage [1,2]. Physical and chemical methods for the removal of hydrocarbons from the environment have some disadvantages compared to biological techniques, including higher cost, lower effectiveness, and toxic effects caused to the environment by chemical compounds such as chemical dispersants. Bioremediation, in turn, arises as a cheaper, more effective, and eco-friendly alternative or complementary technique, which is able to mineralize pollutants or transform them into less toxic substances [3–7]. The choice of the most appropriate and feasible in-situ or ex-situ biological remediation techniques, however, will depend on preliminary analyses of the environmental conditions, type of pollutant, soil composition, removal costs, and time available for treatment. However, the characterization of the contaminated site is the main step in ensuring that bioremediation succeeds [8,9]. Although there are many hydrocarbon-degrading microorganisms in nature, the growth of many of them is hampered by several factors, such as the recalcitrant nature of the substrate and the low availability of organic compounds in aqueous systems, which limits their use as a carbon source [8].

The use of surfactants has become an attractive technology for soil washing in recent years, as hydrophobic pollutants adhere to the surfaces of soil particles, which generally are poorly hydrosoluble. Surfactants are amphipathic agents responsible for the cleaning property of detergent formulations and can be of synthetic or natural origin [9]. Due to their molecular structure, which is made up of polar and apolar moieties, surfactants can solubilize soil contaminants, facilitating their removal [10]. Surfactant molecules increase the solubility of poorly soluble hydrophobic pollutants through emulsification, consequently increasing their availability to the oil-degrading microorganisms during bioremediation [11].

Based on their origin and composition, surfactants can be divided into two main categories: (i) synthetic surfactants and (ii) green surfactants. The surfactants belonging to the first category, which are the most widespread and cheapest ones, are produced synthetically from non-renewable sources, with a final structure different from that of the natural components of living cells. Green surfactants, in turn, comprise both the so-called biobased surfactants and biosurfactants. The first group comprises surfactants with intermediate biocompatibility, which, despite being generally produced by chemical synthesis, contain fats, sugars, or amino acids from renewable sources in their structure, while those of the second group, also called microbial surfactants, are considered the most biocompatible and ecologically safe, being produced by living cells, mainly bacteria and yeasts, without the intermediation of organic synthesis [12].

Biosurfactants show great ability to enhance hydrocarbon solubility and mobility, being attractive for crude oil bioremediation [2,10]. Many studies have described the use of biosurfactants to remediate soils contaminated with organic or inorganic pollutants. A biosurfactant produced from *Pseudomonas aeruginosa*, for example, increased the oil degradation rate by the bacterium by up to 90%, evidencing its potential as an adjunct to stimulate petroleum degradation [13]. The crude biosurfactant produced by *Pseudomonas cepacia* CCT6659 made it possible to recover almost three quarters of the oil from contaminated sand [14]. The 10–20% increase in the rate of oil biodegradation by *Bacillus* sp. and *Candida sphaerica* UCP0995 observed in the presence of their respective biosurfactants suggested that their addition could significantly improve hydrocarbon degradation in the soil [15]. Biosurfactants have been also mixed with inexpensive synthetic surfactants or tested in new formulations to reach a more stable product for the petroleum market [9].

Although many studies have shown that green surfactants are an attractive choice for improving the degradation efficiency of hydrophobic contaminants in soil, it should

be noted that they can also delay or have no effect on the biodegradation of hydrocarbons. The toxicity of green surfactants can cause inhibitory effects on pollutant-degrading bacteria and delay or even inhibit biodegradation. Thus, it is necessary to evaluate new formulations based on green surfactants [10].

Based on this background, the aim of this work was to investigate the tensioactive properties of three green surfactants, i.e., two commercial biobased surfactants and a formulated microbial surfactant produced from the yeast *Starmerella bombicola*, and to apply them in the treatment of soils contaminated with hydrocarbons. The efficiency of the green surfactants was also compared with a synthetic surfactant, and the formulated biosurfactant was applied as a bioremediation agent together with its producing species. Finally, for a potential application in field of these biosurfactants, their ecotoxicity was tested.

2. Materials and Methods

2.1. Materials

All chemicals were of reagent grade. Growth media were purchased from Difco Laboratories (Detroit, MI, USA). The synthetic commercial surfactant Tween 80 (polyoxyethylene 20 sorbitan monooleate), used for comparative purposes, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It is a nonionic surfactant and an oil-in-water emulsifier with a critical micelle concentration (CMC) of approximately 0.0124% (*w*/*v*) (120 mg/L), which allows the water surface tension to be reduced to 43.7 mN/m. Two commercial biobased surfactants, designated as biobased 1 and biobased 2, were tested. The surfactant called biobased 1 was composed of terpenes, surfactants, fatty acid esters, and ethanol (AMBIEVO Ltd., São Paulo, Brazil), while the one, called biobased 2, was composed of esters of fatty acids, natural polymer, and fatty alcohols (IATI, Recife, Brazil). The surface tension of the commercial biobased 1 surfactant was 28.92 ± 0.03 mN/m, while that of the biobased 2 one was 29.70 ± 0.64 mN/m.

Motor oil (15 cSt), i.e., lubricating oil after use, was obtained from an automotive maintenance establishment in the city of Recife, Brazil.

2.2. Yeast Strain and Preparation of Inoculum

The yeast *Starmerella bombicola* ATCC 22214, purchased from American Type Culture Collection (ATCC®) through Plast Labor Ind. Com. Equip. Hosp. Ltd. (Rio de Janeiro, Brazil), was used to produce the biosurfactant. The culture was maintained on Yeast Mold Agar (YMA), which had the following composition: 10 g/L D-glucose, 3 g/L yeast extract, 5 g/L peptone, and 2 g/L agar, pH 7.0.

Yeast Mold Broth (YMB), containing 10 g/L D-glucose, 3 g/L yeast extract, and 5 g/L peptone, pH 7.0, was used for yeast growth. The yeast inoculum was standardized by transferring the young culture to flasks containing 500 mL of the YMB medium and incubating it at 30 °C under orbital shaking at 200 rpm for 48 h. After this period, dilutions were performed to obtain a cell suspension with 10^6 cells/mL final concentration.

2.3. Biosurfactant Production

The biosurfactant was produced in the medium previously described by Konishi et al. [16], consisting of 50 g/L olive oil as a hydrophobic source, 25 g/L glucose, 1 g/L yeast extract, 0.5 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, and 0.3 g/L $NaNO_3$. This medium was modified by replacing olive oil with cotton oil at the same concentration. The components were solubilized and sterilized in an autoclave for 20 min at 121 °C. Fermentations were carried out in 2 L Erlenmeyer flasks containing 1 L of the production medium incubated at 30 °C with 10% (*v*/*v*) of the above pre-inoculum suspension under orbital shaking at 200 rpm for 8 days. Surface tension and pH were determined throughout the fermentation, and biomass concentration was monitored by optical density readings at 600 nm.

2.4. Biosurfactant Isolation

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The biosurfactant produced from *Starmerella bombicola* ATCC 22214 was extracted according to the methodology described by Hu and Ju [17]. The fermentation broth precipitate containing the biosurfactant obtained after 8 days of fermentation was transferred to a separatory funnel and washed twice with 1:1 (*v*/*v*) ethyl acetate. Then, the organic phase was centrifuged at 4000 × *g* for 25 min and filtered through Whatman No. 1 filter paper. The filtrate containing the crude biosurfactant was dried at 40 °C to remove ethyl acetate and gravimetrically quantified.

2.5. Formulation of the Commercial Biosurfactant

The new green detergent formulation based on the biosurfactant from *Starmerella bombicola* ATCC 22214 or each commercial surfactant was formulated with the following components: surfactant (1.0% *w*/*v*), phase stabilizer (hydroxyethyl cellulose 0.4% *w*/*v*), chelating agent (EDTA 1.0% *w*/*v*), and preservative (potassium sorbate 0.2% *w*/*v*). After mixing all components with a mechanical stirrer (Tecnal, Piracicaba, Brazil) at 3000 rpm for 15 min under heating, the formulated detergent sample was kept undisturbed for 24 h and stored in previously sterilized bottles and hermetically closed.

2.6. Evaluation of the Organoleptic Characteristics of the Commercial Biosurfactant

The formulated commercial biosurfactant was subjected to evaluation of its organoleptic characteristics, namely visual changes in color, odor, homogeneity, and consistency, which were classified as follows: color (milky, transparent, or pearlescent), odor (pleasant or unpleasant), consistency (creamy or fluid), and homogeneity (heterogeneous or homogeneous) [18].

2.7. Determination of Surface Tension and Critical Micelle Concentration of the Surfactants

The surface tension and the CMC of the surfactants were determined automatically by a Tensiometer (Sigma 700, KSV Instruments Ltd., Helsinki, Finland), using the Du Noüy ring method, at 28°C.

2.8. Determination of Emulsification Capacity of the Surfactants

The stability of an emulsion is widely used as a surfactant activity indicator. The emulsification index was measured using the method described by Cooper and Goldenberg [19], whereby 3.0 mL of a hydrophobic compound (diesel oil, kerosene, motor oil, *n*-hexadecane, or petroleum) and 3.0 mL of each surfactant were vortexed for 1 min in a test tube. After 24 h, the emulsion index (E_{24}) was calculated according to the formula:

$$E_{24} = E/H \times 100 \quad (1)$$

where E is the measured height of the emulsion layer and H the total height of the mixture, both expressed in cm. Tests were performed in triplicate.

2.9. Application of Surfactants in Hydrophobic Contaminant Spreading

The oil displacement test is a method used to determine the dispersibility of a surfactant, measured as the diameter of the clear region that appears after the surfactant drips in a thin oil onto a water layer. The formed region diameter makes it possible to evaluate the product's efficiency in dispersing slicks of oil derivatives on the water surface. The oil displacement test was carried out by slowly dropping 15 μL of motor oil, diesel oil, or petroleum onto the surface of 40 mL of distilled water in a Petri dish (15 cm in diameter), followed by the surfactant addition in a 1:10 (*v*/*v*) surfactant/oil ratio. The mean diameter of the clear zone formed in the oil surface center was determined visually at room temperature after 30 s, by comparing the obtained value with the negative value of the control (1 mL of distilled water). The larger the formed region diameter, the greater the biosurfactant's surface activity [20,21].

2.10. Toxicity of Surfactants to Artemia salina as an Indicator

Toxicity assays were performed against brine shrimp (the microcrustacean Artemia salina) as a bioindicator using surfactant solutions diluted in distilled water in the proportions of 1:2, 1:5, and 1:10 (*v*/*v*) at final concentrations of 1 and 2%. The larvae were used within 1 day of hatching. The assays were conducted in 15 µL penicillin tubes containing 10 brine shrimp larvae in 10 µL of saline water (33 mg/L) per tube. All larvae were observed for 24 h for the mortality rate calculation [22]. Each test was performed in triplicate.

2.11. Application of Surfactans in Phytotoxycity Tests

The phytotoxicity of the biosurfactant was evaluated in static tests involving the seed germination and root elongation of cabbage (*Brassica oleracea*, var. capitata) and tomato (*Solanum lycopersicum*), according to Tiquia et al. [23]. Test solutions of the surfactants diluted in the proportion of 1:5 (*v*/*v*) in distilled water were prepared and used at a final concentration of 2%. Toxicity was determined in sterilized Petri dishes (10 cm) containing Whatman No. 1 filter paper. After pre-treatment with sodium hypochlorite, ten seeds were inoculated in each Petri dish, followed by the addition of 5 mL of the test solution at 28 °C. After 5 days of incubation in the dark, seed germination, root elongation (≥5 mm), and the germination index (GI), i.e., the factor of relative seed germination and relative root elongation, were determined, as follows:

(1) Relative seed germination (%) = (number of seeds germinated in the extract/number of seeds germinated in the control) × 100.
(2) Relative root elongation (%) = (mean root length in the extract/mean root length in the control) × 100.
(3) GI (%) = (% relative seed germination) × (% relative root elongation)/100.

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2.12. Soils Used in the Removal Experiments

Four soils of different textures, i.e., a sandy, a silty, and a clay soil and beach sand, were used in the experiments. All soils were collected in Pernambuco state, Brazil. The exact geographic regions were as follows: beach sand—Latitude/Longitude 8°05′42.8″ S 34°52′55.3″ W, Pina Beach, Recife city; sandy soil—Latitude/Longitude 7°38′24.9″ S 34°57′21.6″ W, Goiana city; silty soil—Latitude/Longitude 8°23′54.7″ S 35°03′42.2″ W, Ipojuca city, and clay soil—Latitude/Longitude 8°23′53.8″ S 35°03′39.8″ W, Ipojuca city.

Five kg of each soil was stored in nylon bags. Individual samples were then divided into four equal parts in the shape of a cross, with repeated blending of the upper and right arms of the cross with the lower and left arms, respectively, until complete homogenization of samples was achieved. Soils were then left to dry in open air for 4 days and stored until use.

2.13. Characterization of Soils

Physical characteristics of soils were described based on analyses of size distribution [24], liquid limit [25], plasticity [26], specific particle weight [27], and compaction [28], according to the Brazilian Association of Technical Standards (ABNT).

2.14. Application of Surfactants in the Removal of Motor Oil from Packed Columns through the Static Assay

Glass columns measuring 55 cm in height × 6 cm in diameter were initially filled with approximately 100 g of a mixture containing each soil and 10 g of motor oil (15 cSt). The surface was then inundated with 100 mL of the biosurfactant solution under the action of gravity. Percolation of the biosurfactant solution was monitored in 5 min intervals for 24 h, when no further percolation of the solution was observed. The percolation time of the surfactant solution was recorded [29].

2.15. Application of Surfactants in the Removal of Motor Oil from Flasks through the Kinetic Assay

The removal of motor oil from the contaminated soil was carried out through the saturation of 100 g of soil with 10 g of motor oil. The laboratory-contaminated soil was placed in 500 mL Erlenmeyer flasks, to which 100 mL of surfactant solution was added. A flask containing soil and 100 mL of water, with no added surfactant, was used as a control. Flasks were shaken at 50 rpm for 24 h at 28 °C. After treatment, the soil and the washing solution were left to rest for 5 min and separated for analysis. The washing solution was separated with the aid of a pipette, and the remaining soil in the Erlenmeyer was washed with distilled water to remove oil residues from the walls as well as to remove the remnants of the surfactant solution from the soil. This also prevented the formation of an emulsion with the solvent during the subsequent determination of the residual oil in the soil [30].

2.16. Quantification of the Oil Removed in the Static and Kinetic Assays

The initial and final amounts of the hydrophobic contaminants were determined in the aqueous phase (washing solution of the used surfactant) and in the solid phase (soils) after extraction with *n*-hexane. Ten mL of *n*-hexane was added to the aqueous phase and to the soil after washing in a separating funnel, and the solution was shaken for 10 min. This procedure was repeated as many times as necessary until the absorbance of the hexane phase was the same as that of pure hexane (zero absorbance). The final hexane and oil extract was centrifuged at 3000 rpm for 20 min to separate any particles of sand/soil in suspension.

The motor oil removal efficiency was calculated as follows:

a. Oil removed (%) = [initial oil concentration in the soil (g) before washing—final oil concentration in the soil after washing (g)]/[initial oil concentration in the soil (g) before washing] × 100.

b. The removal efficiency was also evaluated by gravimetry, in the same way as described above, after the treatment of the washing solution (surfactant solution containing the contaminant) with hexane.

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2.17. Bioremediation Experiment with the Commercial Biosurfactant from Starmerella bombicola

Soils samples (10 g) contaminated with motor oil were added to 100 mL of drinking water, and the mixture was enriched with 1 mL of sugarcane molasses provided by a local plant. This mixture was previously sterilized under fluent vapor and constituted the control condition. Then, solutions of the commercial biosurfactant at its CMC and at twice its CMC plus 15% of the inoculum containing 10^7 cells/mL in YMB were added to the flasks, and the mixtures were incubated at 150 rpm for 60 days at 28 °C, according to the set of experiments listed in Table 1.

Every 15 days, 1% sugarcane molasses was added to the mixtures, totaling three feedings (after 15, 30, and 45 days). Five mL samples were taken every 15 days up to 60 days for petroleum derivative analysis. The percentage of oil degradation was determined by gravimetry from the concentration of removed oil as follows. Ten mL of *n*-hexane was added to the aqueous phase in a separating funnel, and the solution was shaken for 10 min. The procedure was repeated twice, and the final extract containing hexane and oil was heated in an oven at 68–70 °C to evaporate the hexane and quantify the residual oil.

Table 1. Formulated mixtures for motor oil biodegradation experiments. CMC = critical micelle concentration.

Experiment	Composition
Set 1	Contaminated oil + sugarcane molasses + *S. bombicola*
Set 2	Contaminated oil + sugarcane molasses + *S. bombicola* + biosurfactant at the CMC
Set 3	Contaminated oil + sugarcane molasses + *S. bombicola* + biosurfactant at twice the CMC
Control	Contaminated oil + sugarcane molasses

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2.18. Statistical Analysis

All determinations were performed in triplicate. Means and standard deviations were calculated using Microsoft Office Excel 2016. A 95% confidence interval and a 5% significance level were considered; p-values < 0.05 were statistically significant.

3. Results and Discussion

3.1. Production of the Commercial Biosurfactant from Starmerella bombicola

3.1.1. Production and Isolation of the Biosurfactant

The biosurfactant production using *Starmerella bombicola* ATCC 22214 was proven by the reduction of surface tension to 32.30 mN/m in 192 h of fermentation, meaning a 77.6% reduction in relation to that of distilled water (72 mN/m), while cell growth was evidenced by an increase in the optical density (O.D.) at 600 nm wavelength up to 2.892 and a pH decrease to 5.3 at the end of fermentation (Table 2).

Table 2. Values of surface tension, optical density, and pH during the cultivation of *Starmerella bombicola* ATCC 22214 for 192 h. Results are expressed as means ± SD ($n = 3$), where means are significant at $p < 0.05$.

Time (h)	Surface Tension (mN/m)	Optical Density at 600 nm	pH
0	72.00 ± 0.2	0.576 ± 0.2	6.0 ± 0.3
24	45.67 ± 0.1	1.619 ± 0.5	5.2 ± 0.1
48	37.03 ± 0.5	2.548 ± 0.3	4.6 ± 0.1
72	35.01 ± 0.5	2.765 ± 0.1	4.5 ± 0.2
96	34.27 ± 0.5	2.835 ± 0.1	5.0 ± 0.5
120	33.87 ± 0.3	2.838 ± 0.5	5.2 ± 0.5
144	33.45 ± 0.2	2.857 ± 0.3	5.2 ± 0.2
168	32.52 ± 0.1	2.888 ± 0.2	5.4 ± 0.3
192	32.30 ± 0.1	2.892 ± 0.2	5.3 ± 0.1

The isolated biosurfactant showed a yield of 32.5 g/L and a CMC of 0.65 g/L (Figure 1). Sharma and Sharma [31], who optimized the production of biosurfactants (by response surface methodology, Plackett–Burman, and others) in bioreactors under different cultivation conditions, reported maximum yields ranging between 8.5 and 58 g/L depending on the microorganism, namely *Bacillus subtilis* E8 (20 g/L), *Candida bombicola* (61 g/L), *Candida tropicalis* UCP0996 (36 g/L), and *Candida lipolytica* UCP 0988 (40 g/L). Since no optimization effort was made in this study, the yield obtained under the tested conditions can be considered rather promising, especially compared to results described in the literature for microbial surfactants [1,2,9].

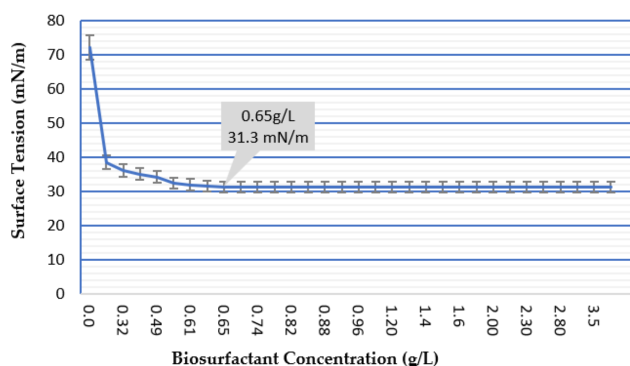

Figure 1. Determination of critical micelle concentration of the biosurfactant produced from *Starmerella bombicola* ATCC 22214.

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Corroborating the results obtained here, Luna et al. [32] produced a biosurfactant from *C. bombicola* URM 3718 in a medium based on distilled water with 5% corn steep liquor, 5% molasses, and 5% residual soy frying oil for environmental applications. The *C. bombicola* biosurfactant showed excellent surface tension reduction capacity (from 70 to 30 mN/m) with a CMC of 0.5 g/L. These results demonstrate that the biosurfactant produced by *S. bombicola* ATCC 22214 has a capacity to reduce the surface tension close to that of biosurfactants from *C. lipolytica* (32 mN/m) [33] and *Candida glabrata* (31 mN/m) [34] and higher than that from *Candida antarctica* (35 mN/m) [35], *Yarrowia lipolytica* (50 mN/m) [36], and *C. bombicola* (39 mN/m) [37].

3.1.2. Formulation of the Commercial Biosurfactant

Detergents are a mixture of surfactants with cleaning properties in dilute solutions. Most commercial detergents used to remove oils and greases are derived from crude oil, whose supply is limited [38]. Therefore, biodegradable and non-toxic materials have been investigated as substitutes for these compounds [9].

According to the results illustrated in Figure 2, the formulation of the green detergent based on the biosurfactant isolated from *S. bombicola* ATCC 22214 showed stability after one month of testing; that is, there was no phase separation or change in its organoleptic properties, keeping its pearlescent color, pleasant odor, fluidity, and homogeneous consistency.

Rocha e Silva et al. [39] formulated a non-toxic biodetergent capable of removing oily residues generated during industrial processes, which proved to be stable and able to remove up to 100% of heavy oils from metal surfaces. The formulation composed of cottonseed oil as a natural solvent, saponin as a vegetable surfactant, and carboxymethylcellulose and glycerin as non-toxic stabilizing agents was very promising from an economic point of view, considering the costs of the formula components. Almeida et al. [40], who studied the characterization and commercial formulation of a biosurfactant from *C. tropicalis* UCP0996 and its application in the decontamination of petroleum pollutants, reported a formulation with potassium sorbate that showed stability and promoted a high emulsification rate of the motor oil (above 90%) under practically all conditions tested.

Figure 2. Illustration of the process for obtaining the biosurfactant from *Starmerella bombicola* ATCC 22214: (**A**) culture medium containing the biosurfactant produced and isolated in a separating funnel; (**B**) isolated biosurfactant, and (**C**) commercial biosurfactant formulation.

3.2. Emulsification Capacity of the Surfactants

The stability of an emulsion is indicative of a biosurfactant's surface activity, although the emulsification capacity is unrelated to its ability to reduce surface tension [41,42]. The emulsification index (E_{24}) is a rapid method for evaluating the emulsifying properties of surfactants. The results obtained for the capacity of the surfactants of emulsifying different hydrophobic substrates are listed in Table 3.

The three green surfactants emulsified satisfactorily the oils studied when compared to the synthetic surfactant (Tween 80).

Table 3. Emulsification indexes for different hydrocarbons of the surfactants tested. Results are expressed as means ± SD (n = 3), where means are significant at $p < 0.05$.

Surfactant	Emulsification Index (%)					
	Diesel Oil	**Kerosene**	**Motor Oil**	***n*-Hexadecane**	**Petroleum**	**Global Mean**
Biobased 1	57.9 ± 3.6	56.6 ± 1.2	51.7 ± 2.5	57.7 ± 1.5	61.1 ± 7.7	57.0 ± 4.6
Biobased 2	24.9 ± 2.3	25.8 ± 3.8	98.6 ± 2.3	28.8 ± 6.1	66.6 ± 3.3	48.9 ± 3.5
Commercial biosurfactant	55.0 ± 0.5	75.3 ± 0.3	100.0 ± 0.0	64.1 ± 1.0	99.0 ± 0.3	87.5 ± 0.5
Tween 80	0.0 ± 0.0	0.0 ± 0.0	73.1 ± 0.03	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

In particular, the biobased 1 surfactant had an almost constant emulsification efficiency, regardless of the substrate, while the biobased 2 surfactant emulsified especially motor oil and petroleum, showing good affinity for heavier oils, and the chemical one (Tween 80) only motor oil. The commercial biosurfactant from *S. bombicola* exhibited the greatest emulsifying potential when compared to the other surfactants. These results show that biosurfactants from yeasts have the potential to replace synthetic surfactants, especially in the petroleum industry [43]. The lowest emulsification capacity of the commercial biosurfactant was observed towards diesel oil. The inability of *C. bombicola* sophorolipids to form stable emulsions with diesel oil is confirmed in the literature and seems to be related to the molecular conformation of the two compounds used in the emulsification mixture [44]. Considering that an emulsification index above 50% is very satisfactory for a surfactant molecule [2], the findings show the feasibility of the green surfactants tested here for industrial applications.

The ability of *S. bombicola* biosurfactants to emulsify vegetable oils has also been described in the literature [41,45]. Sophorolipids have a high surface and interfacial activity, which contribute to the emulsification action [46]. Sen et al. [47], using a new yeast strain,

Rhodotorula babjevae YS3, in Bushnell-Haas medium containing glucose (10% *w*/*v*) as the sole carbon source, observed a reduction in the surface tension of the culture medium to 32.6 mN/m and 100% emulsification index for crude oil. Elshafie et al. [48], using *C. bombicola* to produce biosurfactants from media with different carbon sources (glucose (2% *w*/*v*) and corn oil (10% *v*/*v*)), reported emulsification indexes of 68.75% for heavy crude oil and lower values (23.86–29.55%) for the other tested substrates (*n*-hexadecane, light crude oil, *n*-tetradecane, *n*-pentane, and *n*-tridecane). Almeida et al. [40] also produced a *C. tropicalis* biosurfactant UCP0996 in a low-cost medium formulated with molasses, residual frying oil, and corn steep liquor, with high capacity to emulsify motor oil (above 70%).

3.3. Application of the Surfactants in Hydrophobic Contaminant Spreading

The oil displacement test is an indirect measure of the potential ability of a surfactant to disperse oils. The larger the diameter of the clear zone formed, the greater the potential of the surfactant [20,21]. Figure 3 shows the diameters of the clear zones, on the oil surface, produced in the displacement test on the three types of oils (diesel oil, motor oil, and petroleum) by the studied surfactants.

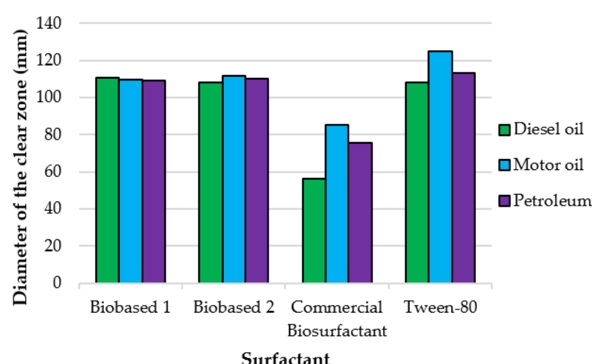

Figure 3. Diameters of the clear zones, on the oil surface, produced in the displacement test on the three types of oils by the tested surfactants. Results are expressed as means ± SD ($n = 3$) where means are significant at $p < 0.05$.

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The analysis of the results allows us to affirm that the two biobased surfactants and the chemical surfactant (Tween 80) showed a better dispersion capacity than the biosurfactant from *S. bombicola* ATCC 22214. Nonetheless, the dispersion capacity of the latter is consistent with the values found in the literature for promising biosurfactants produced from yeasts. Andrade et al. [49] found that a biosurfactant produced from *Cunninghamella echinulata* in a medium containing corn steep liquor (2%) and residual frying oil (0.5%) supplemented with 2% instant noodle waste behaved as an effective dispersant agent, being able to create a clear zone diameter of 63.9 mm on engine motor oil. Surpassing this expectation, the clear zone diameter on motor oil caused by the biosurfactant from *S. bombicola* was 85.5 ± 3.2 mm. Almeida et al. [40] also evaluated the ability of a biosurfactant from *C. tropicalis* to disperse motor oil, obtaining satisfactory removal (71%).

3.4. Toxicity of the Surfactants to Artemia salina

The absence of toxicity of a biosurfactant is essential for its application in the environment; therefore, ecotoxicity bioassays are used as analytical methods to characterize the toxicity of chemical substances. For many decades, species of aquatic crustaceans belonging to the *Artemia* genus have been considered very efficient, versatile, short-lived,

easy-to-handle, and low-cost indicators of toxicity [50,51], which has enabled their application in toxicological studies worldwide [52]. In addition to being bioindicators in contaminated environments, they are used to test the toxicity of a wide range of chemicals and compounds, pesticides, engineered nanomaterials, antibiotic drugs, anti-biofilm agents, anti-corrosive agents, medicines, food products, and oil dispersants [53,54].

The commercial biosurfactant produced by *S. bombicola* ATCC 22214 and the biobased 2 surfactant did not prevent the hatching and life of the *Artemia salina* larvae, regardless of their concentration, demonstrating that they are not toxic for this bioindicator (Table 4). Similar results were found by Almeida et al. [40], who tested the toxicity of the biosurfactant from *C. tropicalis* UCP0996. Dos Santos et al. [55] also demonstrated the absence of any toxicity of a biosurfactant from *C. lipolytica* even at the highest concentration. This comparison demonstrates the low toxicity or even lack of toxicity of biosurfactants produced from yeasts. Regarding the biobased 1 surfactant, higher concentrations promoted the death of the microcrustacean (1 and 2%, *v*/*v*), as also shown in Table 4. The chemical surfactant Tween 80 also showed some toxicity, confirming the results of Sahgal et al. [56] on *A. salina* using Tween 80 solutions at different concentrations.

Table 4. Survival rate (%) of *Artemia salina* larvae in the presence of surfactant solutions diluted in the 1:2, 1:5, and 1:10 (*v*/*v*) of surfactant/distilled water ratios and used at concentrations of 1 or 2%.

Surfactants	**Surfactant/Distilled Water Ratio (*v*/*v*), and Concentration Tested (%)**					
	1:2, 1	**1:2, 2**	**1:5, 1**	**1:5, 2**	**1:10, 1**	**1:10, 2**
Biobased 1	10.0 ± 1.6%	0.0 ± 0.0%	90.0 ± 1.0%	83.0 ± 1.0%	100.0 ± 0.0%	100.0 ± 0.0%
Biobased 2	93.0 ± 0.6%	97.0 ± 1.2%	90.0 ± 1.0%	95.0 ± 0.6%	90.0 ± 1.0%	93.0 ± 1.2%
Commercial biosurfactant	100.0 ± 0.0%	90.0 ± 1.0%	93.0 ± 0.6%	97.0 ± 0.6%	77.0 ± 0.65	80.0% ± 0.1%
Tween 80	47.0 ± 0.6%	50.0 ± 1.0%	90.0 ± 1.0%	77.0 ± 1.0%	80.0 ± 10.1%	90.0 ± 1.0%

3.5. Toxicity of the Surfactants towards Vegetables

The exposure of living organisms (bioindicators) to chemical substances is a valuable tool for environmental analysis [57]. The use of plants in toxicity tests offers several advantages, including low maintenance costs and quick results, as well as a detailed evaluation of potentially toxic compounds for the terrestrial ecosystem [58].

Figure 4 shows the dependence of the germination index (GI) (%) on the type of surfactant and the seed used as toxicity bioindicators. Using the biosurfactant from *S. bombicola* ATCC 22214, the GI was 88.70 ± 0.58% for *Brassica oleracea* seeds and 87.90 ± 0.54% for *Solanum lycopersicum* seeds. The superiority in this aspect of the commercial biosurfactant compared to the biobased 1 surfactant and Tween 80 is noticeable. In fact, the biosurfactant had no phytotoxic effect on either of the seed types, since a GI > 80% is considered an indicator of the absence of phytotoxicity, while values between 50 and 80% point to moderate phytotoxicity [23]. Among the surfactants, Tween 80 showed toxicity towards the seeds of two tested horticultural crops, while both the commercial biosurfactant and the biobased 2 surfactant showed excellent results, allowing GI > 90% for both. Gálvez et al. [59] determined the effect of synthetic surfactants on germination and root elongation.

Several biosurfactants produced from yeasts of the *Candida* genus have been studied for toxicity. Lira et al. [60] evaluated the toxicity of a biosurfactant from *C. guilliermondii* in a short bioassay. The results revealed that the tested solutions had no inhibitory effect on seed germination or root growth, indicating no toxicity of the biosurfactant. Similar results were obtained by Luna et al. [61] and Rufino et al. [62], who assessed the toxicity of a biosurfactant produced from *Candida* spp. from renewable substrates in submerged fermentation. Rocha e Silva et al. [39] developed a high-efficiency biodetergent formulation, capable of cleaning residual oils generated in industrial processes, with toxicity rates as low as that of the studied biosurfactant (88.9 ± 0.21% for *B. oleracea* seeds and 92.70 ± 0.14% for *S. lycopersicum* seeds). It is also important to mention that secondary root leaf

growth was observed under all conditions tested (92.6 ± 0.6 mm for *B. oleracea* seeds 71.8 ± 0.5 mm for *S. lycopersicum* seeds). Similar values for *B. oleracea* root length in control trials (93.5 ± 0.85 mm) were reported by Kage et al. [63], who measured and modeled *B. oleracea* root growth under unstressed conditions, and by Al-Mharib et al. [64], who studied the growth and production indicators for *B. oleracea* treated with mineral fertilizers and root improvers. Similarly, Abdel-Farid et al. [65], studying the effect of salinity stress on the growth and metabolomic profile of *Cucumis sativus* and *S. lycopersicum*, observed values close to those of the control trials of this study with *S. lycopersicum* seeds (71.1 ± 0.75 mm).

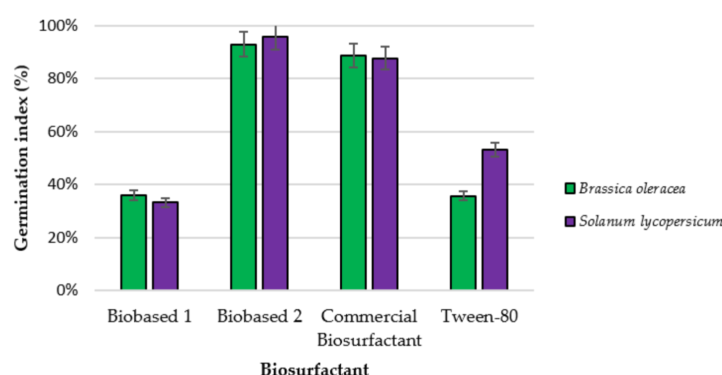

Figure 4. Germination index (%) of *Brassica oleracea* and *Solanum lycopersicum* seeds in the presence of the surfactants. Error bars represent the standard deviation of the results.

3.6. Characterization of Soils

Table 5 shows the results of granulometry, consistency limit, and compaction tests on the four soils under investigation. The compaction test used the standard energy of the Normal Proctor.

The silty soil is the most plastic of all, since its plasticity index (PI) value (26%) indicates a high-plasticity silt. The other samples are moderately plastic, except beach sand, which is non-plastic (NP). The silty soil, collected from the top of a slope, has an open particle size, indicating that it is a leached soil. From the analysis of the granulometric composition of the soil, it is observed that there is a large number of fines in the sample, with more than 50% of the material passing through the sieve # 200 (0.075 mm). Regarding the activity index (Ia), the silty soil can be classified as normal according to Skempton's criterion [66]. The other samples can be classified as inactive, having an Ia value < 0.75.

Table 5. Granulometry, consistency, and unified classification of soils.

Parameters	Soil			
	Beach Sand	Sandy	Silty	Clay
Granulometry (%)				
Sand	98	56	30	26
Silt	1	5	39	30
Clay	1	39	31	44
% Liquidity–plasticity (LP)< 2 µm	0.6	37	45	53
Consistency				
Liquid limit (%)	0	47	71	66
Plasticity index (PI) (%)	0	10	26	13
I_a [1]	0	0.29	1.0	0.59

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Compaction				
Optimum moisture (%)	6.5	34	26	22
μ_{dmax} [2] (kN/m^3)	14.7	26.5	27.6	26.8
Unified classification	SP	SC	MH	CH

[1] I_a: activity index. I_a = PI/≤2 μm—activity. [2] μ_{dmax}: maximum apparent specific dry weight.

According to the Unified Soil Classification System (USCS), the sandy sample is designated as SC (clay sand), the silty sample as MH (high-plasticity silty soil), the clay sample as CH (inorganic clay of high compressibility), and the beach sand as SP (poorly graded sand). According to Pastore and Fontes [67], soils in the CH group are expected to have low permeability and low drainage. As expected, beach sand does not present limits of liquidity–plasticity, and the optimum moisture value of this sand is low compared to the others.

3.7. Application of Surfactants in the Removal of Motor Oil Adsorbed on Soils

The use of surfactants has become an attractive technology for soil washing in recent years since hydrophobic contaminants adhere to the surfaces of soil particles and generally have reduced water solubility. Thus, due to their molecular structure, surfactants can be added to solubilize soil contaminants. A wide variety of green surfactants, especially biosurfactants, have already been applied for soil remediation at laboratory level [68].

Soil washing using surfactants can be carried out ex situ and in situ. For ex-situ washing, the contaminated excavated soil is pre-treated and mixed with the solution containing surfactants and agitated. After washing, the clay particles are deposited, and the washing solutions can be separated and regenerated for use in the next round. In-situ washing of the soil with surfactant eluents is another strategy for practical application. Washing surfactant solutions are injected into the contaminated area through injection wells. Soil contaminants are mobilized by solubilization (for example, formation of micelles with the help of washing solutions) or chemical interactions. After passing through the contaminated zone, the fluid containing the contaminants is collected and brought to the surface for disposal, recirculation, or treatment and reinjection in place [10].

3.7.1. Removal of Motor Oil from Packed Columns through the Static Assay

Surfactants help in bioremediation processes, as they can increase the mobility and bioavailability of hydrocarbons [69]. The hydrophobic portion of the surfactant chemically binds to the hydrophobic coating of the soil particle, making it wettable. Simultaneously, the hydrophilic head attracts water molecules, allowing them to pass through the soil and increase infiltration [70].

Figure 5 shows that the commercial biosurfactant produced by *S. bombicola* ATCC 22214 showed the highest average index for motor oil removal in packed columns. The highest values were observed for sandy soil (80.0 ± 0.46%) and beach sand (65.0 ± 0.14%), likely because sandy materials have high permeability, facilitating percolation. However, tests carried out with clay and silty soils did not show high removal of the contaminant.

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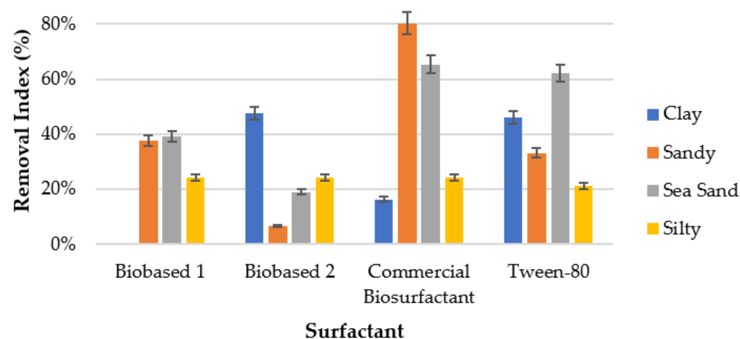

Figure 5. Removal of motor oil adsorbed to four types of soils in packed glass columns (static assay) using the four surfactants tested.

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In general, the tested surfactants were not successful in removing the contaminant from the clay soil due to some soil characteristics, such as high liquid retention and low permeability. In fact, this type of soil has very small particles (micropores), and, since the spaces among grains (pores) are also very small, they retain more water, making it saturated [71]. This may have prevented the complete percolation of the surfactants and, consequently, led to the low removal of the contaminant. This result is evident in Figure 6, where it is possible to recognize the low percolated volume of clay soil compared to the other soils. Figure 7 illustrates the appearance of soil samples before contamination, after contamination with motor oil, and washed with the commercial biosurfactant.

The silty soil always showed a low removal rate regardless of the surfactant used (20 to 22%). Moreover, in addition to behaving similarly to clay soil, it easily turned into mud due to its plasticity [72]. It is likely that the contaminant was retained in this soil, which made its removal by the surfactants difficult.

In Figure 6, it can also be noted that, regardless of the soil used, Tween 80 was retained in the packed columns (low percolated volume), likely due to its high viscosity. Similar results were found by Chaprão et al. [15], who reported 45.0 ± 2.0% motor oil removal from sand in packed columns using Tween 80. The results show that all surfactants removed mainly the oil adsorbed on the sand compared to the control (distilled water), whose removal, on the other hand, is related to the gravitational and mechanical action of the discharge [13].

Similar results were obtained by Rufino et al. [29], who studied the removal of motor oil adsorbed on three types of soil by the biosurfactant Rufisan produced by *C. lipolytica* UCP0988. The oil removal indexes obtained were 31.2 ± 0.4% in clay, 33.1 ± 0.5% in sand, and 30.0 ± 0.6% in silt. Chaprão et al. [15] reported similar removal percentages using *Bacillus* sp. and *Candida sphaerica*. Jimoh and Lin [73] obtained 73% removal of engine oil with the biosurfactant produced by *Paenibacillus* sp. D9, performing better than the chemical surfactant SDS (58%). The *Pseudomonas aeruginosa* UCP0992 biosurfactant, on the other hand, showed removal rates of approximately 80% when cell-free fermented broth was used, but only less than 60% when using the purified biosurfactant [13].

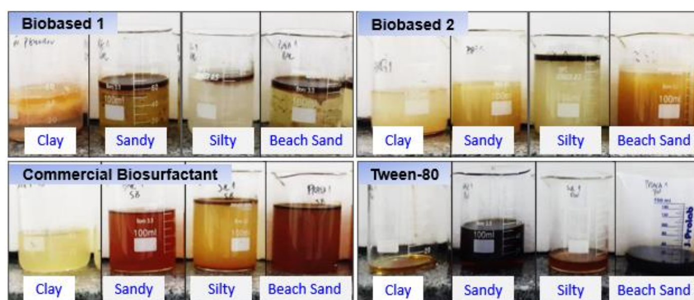

Figure 6. Illustrations showing the tested surfactant washing solutions (biobased 1, biobased 2, formulated biosurfactant, and Tween 80) after hydrocarbon removal through static assay from soils (clay, sandy, silty, and beach sand).

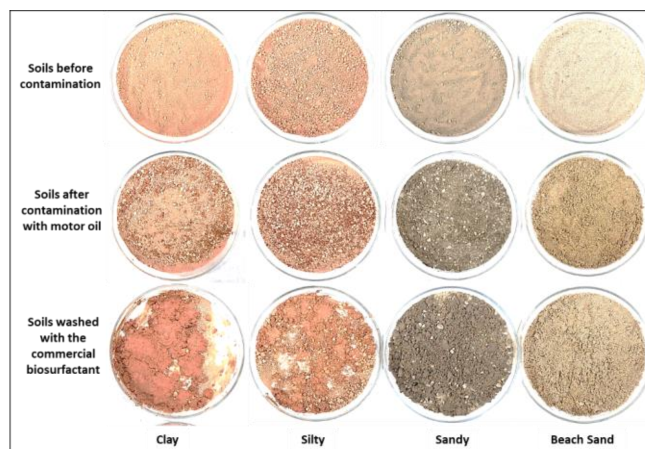

Figure 7. Illustration showing appearance of the soil samples before contamination, after contamination with motor oil, and washed with the formulated biosurfactant applied in static assay.

Durval et al. [54], using a biosurfactant produced from *Bacillus cereus* UCP1615 as an environmental remediation agent, obtained for an oil adsorbed on sand a lower removal index (63.0 ± 2.1%) than that obtained with the *S. bombicola* ATCC 22214 biosurfactant (80.0 ± 0.46%), which highlights the ability of the commercial biosurfactant in removing hydrophobic contaminants.

3.7.2. Removal of Motor Oil from Flasks through the Kinetic Assay

One of the main obstacles to the bioremediation of contaminants in soil is the ability of petroleum hydrocarbons to bind to soil particles physically and chemically, thus hindering the removal and degradation of these compounds [74].

The commercial biosurfactant and biobased 2 surfactant showed the highest removal indexes for motor oil adsorbed on the four soil types in the kinetic assay (Figure 8). As in the static column tests, due to the characteristics of clay and silty soils (low permeability and high plasticity values), there was a low capacity to remove the contaminant in these soils. However, the kinetic test allowed a greater removal of motor oil when compared to static tests—that is, a 26% increase with the biobased 2 surfactant from sandy soil, an 8%

increase with the commercial biosurfactant from sandy soil, a 43% increase with the biobased 2 surfactant from silty soil, and a 32% increase with the commercial biosurfactant from silty soil. Such a generalized increase in removal can be explained by the greater interaction between the contaminated soil and the surfactant, promoted by agitation.

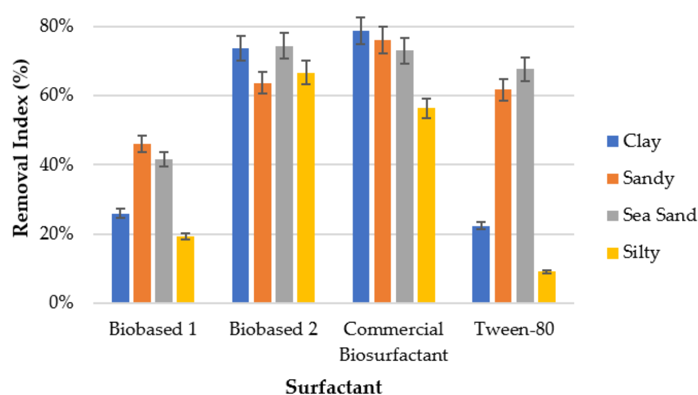

Figure 8. Removal of motor oil adsorbed to four types of soils in flasks (kinetic assay) using the four surfactants tested.

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Due to the greater permeability of sand, higher removal yields compared to clay and silt were expected; however, there seems to have been a good interaction between the biosurfactant and clay/silt, which improved removal. The chemical surfactant Tween 80 showed a good contaminant removal capacity in sandy soils—that is, in beach sand (68.0 ± 2.1%) and in common sand (62.0 ± 1.2%)—but, due to its high viscosity, it did not give satisfactory values for silty (40.0 ± 2.1%) and clay (22.0 ± 1.4%) soils. The biobased 1 surfactant had the lowest mean values for motor oil removal.

Figure 9 shows experimental tests in which liquids (surfactant + contaminant) were separated from the soils for evaporation of *n*-hexane and quantification of removed motor oil. The smaller the volume of liquids in the beakers, the greater the interaction between surfactants and soils. It is evident that there was an interaction between Tween 80 and the soil, due to the high viscosity of this surfactant. Figure 10 illustrates the appearance of the soil samples before contamination, after contamination with motor oil, and washed with the commercial biosurfactant.

A similar removal index (85%) was reported by Silva et al. [75] in kinetic tests to remove diesel oil adsorbed on sand using a biosurfactant produced from *P. aeruginosa*, while the motor oil removal capacity was much lower (20%). Chaprão et al. [15] obtained 70% engine oil removal from sand in a kinetic test using Tween 80.

Figure 9. Illustrations showing the tested surfactant washing solutions (biobased 1, biobased 2, formulated biosurfactant, and Tween 80) after hydrocarbon removal through kinetic assay from soils (clay, sandy, silty, and beach sand).

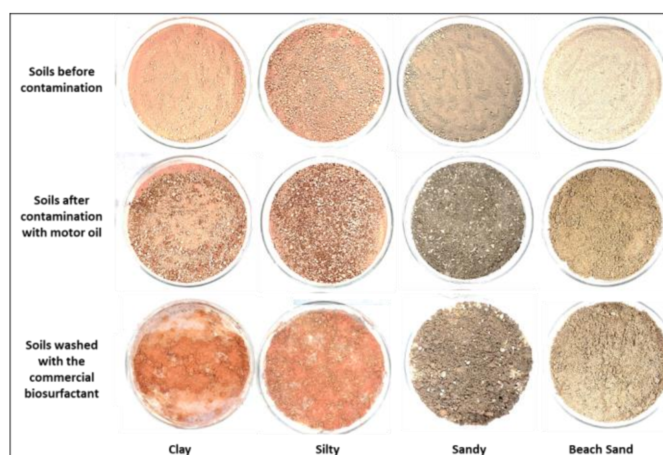

Figure 10. Illustration showing appearance of the soil samples before contamination, after contamination with motor oil, and washed with the formulated biosurfactant applied in kinetic assay.

The results obtained for the commercial biosurfactant were close to those of Rufino et al. [29], who studied the removal of motor oil adsorbed in three types of soil by a biosurfactant produced from *C. lipolytica* UCP0988. The removal indexes in the kinetic tests were 98.0 ± 0.4% from clay, 98.0 ± 0.6% from sandy, and 98.1 ± 1.0% from silty soil. Durval et al. [54], using a biosurfactant produced from *B. cereus* UCP1615, obtained a removal yield of 84% in kinetic assays from beach sand. The results of contaminant removal from clay soil found using the commercial biosurfactant (79.0 ± 2.8%) and the biobased 2 surfactant (74.0 ± 2.3%) were quite satisfactory in the kinetic assay. Rocha and Silva et al. [74], using a *Pseudomonas* biosurfactant, also obtained satisfactory motor oil removal from clay soil, with removal rates above 80%.

3.8. Influence of the Commercial Biosurfactant from Starmerella bombicola on the Bioremediation of Soil Contaminated with Motor Oil

Three sets of experiments were carried out to study motor oil biodegradation, whose results, collected after 15, 30, 45, and 60 days, are shown in Figure 11. For this purpose, molasses was added to the contaminated soil mixtures under all experimental conditions in order to provide the necessary nutrients for both microbial growth and the

biodegradation of the petroleum derivative. In fact, molasses is an abundant co-product of sugar production, both from sugarcane and from the sugar beet industry in Brazil, which are rich in carbon, organic nitrogen, and mineral compounds.

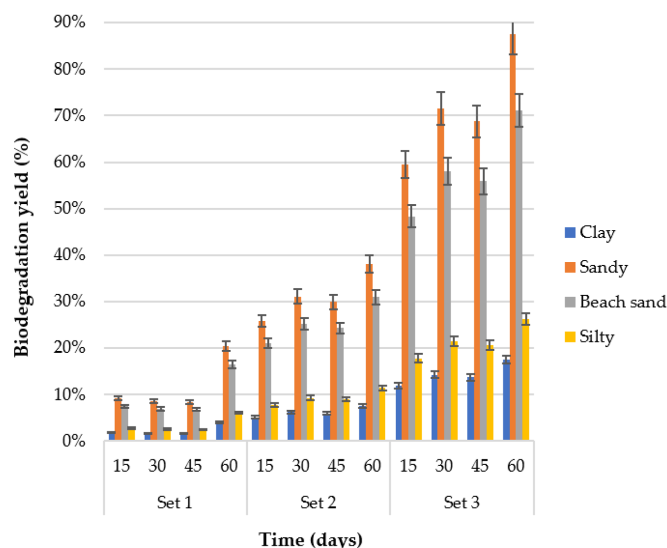

Figure 11. Yield of motor oil biodegradation. Set 1: contaminated soil + sugarcane molasses + *S. bombicola*; Set 2: contaminated soil + sugarcane molasses + *S. bombicola* + biosurfactant at CMC; Set 3: contaminated soil + sugarcane molasses + *S. bombicola* + biosurfactant at twice the CMC. Error bars show the corresponding standard errors.

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The experiments carried out on sandy soil and beach sand showed the highest yield of biodegradation. In the first set of experiments (Set 1: contaminated soil + sugarcane molasses + *S. bombicola*), oil degradation reached a yield of 20.3 ± 1.2% after 60 days using the sandy soil. In the presence of the commercial biosurfactant at CMC (Set 2), the yield increased during the first 30 days, reaching values around 38.1 ± 1.9% after 60 days. Using the commercial biosurfactant at twice the CMC (Set 3), the biodegradation yield increased to 88% ± 3.8% after 60 days. The biosurfactant enhanced hydrocarbon biodegradation by yeast probably due to i) an increase in the surface area of water-insoluble hydrophobic substrates and ii) an increase in the bioavailability of hydrophobic compounds [76]. Biodegradation in silty and clayey soils was lower, since, even with the commercial biosurfactant at the highest concentration, the maximum yield was only 26.0 ± 1.2% after 60 days.

Most studies describe the use of bacteria in the degradation of petroleum hydrocarbons, although the efficiency of yeasts has also been demonstrated. The effectiveness of *Candida catenulata* CM1 in degrading petroleum hydrocarbons was evaluated during the composting of a mixture containing 23% food waste and 77% diesel-contaminated soil. After 13 days of composting, 84% of the initial petroleum hydrocarbons was degraded [77].

The results of these experiments confirm the action of commercial biosurfactants as a facilitator of motor oil degradation. In a similar study involving the degradation of motor oil adsorbed onto sand using *C. sphaerica*, Chaprão et al. [15] reported degradation yields of 20–25% for the microorganism alone and of 35–40% with the addition of the *C. sphaerica* biosurfactant.

4. Conclusions

The tested green surfactants exhibited specificity for each application, generally demonstrating promising results as removers of hydrophobic contaminants. The commercially formulated biosurfactant produced from *Starmerella bombicola* considerably reduced the surface tension of water, demonstrating a high capacity to emulsify and disperse hydrophobic compounds and an absence of toxicity under the conditions tested. The formulated biosurfactant also stood out in removing motor oil in static and kinetic tests, in addition to increasing the contaminant's biodegradation in different types of soils. The results obtained in this study demonstrate the feasibility of formulating new commercial biosurfactants with prospects for industrial application.

It is important to remember that increasing awareness and the need for environmental protection are driving researchers to look for eco-friendly products and pave the way for the development of clean and safe surfactants using renewable resources. Laboratory- and pilot-scale chemical, enzymatic, and microbial syntheses have already increased the production and structural diversification of green surfactants, with notable benefits. Although these surfactants are promising alternatives to their synthetic counterparts, their commercialization is still limited. Future research will ensure the replacement of chemical surfactants, leading to improvements at all levels, including performance, cost-effectiveness, and environmental compatibility.

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