Removal of heavy oil from contaminated surfaces with a detergent formulation containing biosurfactants produced by *Pseudomonas* spp.

Charles Bronzo B. Farias[1,2], Rita de Cássia F. Soares da Silva[2,3], Fabíola C.G. Almeida[2],
Valdemir A. Santos[1,2,3], Leonie A. Sarubbo[1,2,3]

[1] Rede Nordeste de Biotecnologia (RENORBIO), Universidade Federal Rural de Pernambuco, Rua Dom
Manuel de Medeiros, s/n, dois Irmãos, CEP: 52171-900, Recife, Pernambuco, Brasil
[2] Instituto Avançado de Tecnologia e Inovação (IATI), Rua Potyra, n. 31, Prado, CEP: 50751-310, Recife,
Pernambuco, Brasil
[3] Universidade Católica de Pernambuco (UNICAP), Rua do Príncipe, n. 526, Boa Vista, CEP: 50050-900,
Recife, Pernambuco, Brasil

Corresponding Author:
Leonie Sarubbo[1,2,3]
Rua do Príncipe, n. 526, Boa Vista, CEP: 50050-900, Recife, Pernambuco, Brasil
Email address: leonie.sarubbo@unicap.br

Abstract

Industrial plants powered by heavy oil routinely experience problems with leaks in different
parts of the system, such as during oil transport, the lubrication of equipment and mechanical
failures. The surfactants, degreasing agents and solvents that make up detergents commonly used
for cleaning grease-covered surfaces are synthetic, non-biodegradable and toxic, posing risks to
the environment as well as the health of workers involved in the cleaning process. To address
this problem, surfactant agents of a biodegradable nature and low toxicity, such as microbial
surfactants, have been widely studied as an attractive, efficient solution to replace chemical
surfactants in decontamination processes. In this work, the bacterial strains *Pseudomonas
cepacia* CCT 6659, *Pseudomonas aeruginosa* UCP 0992, *Pseudomonas aeruginosa* ATCC 9027
and *Pseudomonas aeruginosa* ATCC 10145 were evaluated as biosurfactant producers in media
containing different combinations and types of substrates and under different culture conditions.
The biosurfactant produced by *P. aeruginosa* ATCC 10145 cultivated in a mineral medium
composed of 5.0% glycerol and 2.0% glucose for 96 h was selected to formulate a biodetergent
capable of removing heavy oil. The biosurfactant was able to reduce the surface tension of the
medium to 26.40 mN/m, with a yield of approximately 12.00 g/L and a critical micelle
concentration of 60.00 mg/L. The biosurfactant emulsified 97.40% and dispersed 98.00% of the
motor oil. The detergent formulated with the biosurfactant also exhibited low toxicity in tests
involving the microcrustacean *Artemia salina* and seeds of the vegetable *Brassica oleracea*. The
detergent was compared to commercial formulations and removed 100% of the Special B1 Fuel
Oil (OCB1) from different contaminated surfaces, demonstrating potential as a novel green
remover with industrial applications.

Introduction

44 Fuel and lubricant spills that occur in industries during the filling machines and storage tanks and
45 the washing of equipment are one of the causes of the accumulation of petroleum byproducts in
46 the environment. For engines contaminated with lubricating oil, the removal of adhered grease
47 poses a different challenge from contamination generated by oily sludge, as the cleaning process
48 requires the direct application of a detergent, surfactant or solvent, often generating further
49 environmental problems due to the toxicity and accumulation of these substances (*Rocha e Silva*
50 *et al., 2019*).
51   Detergents are generally comprised of a mixture of surfactants (nonionic and anionic,
52 constituting 1–50%) plus volatile organic solvents, stabilizers, and other additives capable of
53 reducing the surface/interfacial tension of water, though exact compositions are proprietary
54 information (*Thomas et al., 2021*). For instance, the Corexit dispersants are a mixture of
55 hydrocarbons (60–100%), organic sulfonic acid salts (10–30%), and propylene glycol (1–5%)
56 (*Rongsayamanont et al., 2017*). Solvents are used to reduce the viscosity of surfactants, to dilute
57 the detergent, to lower its freezing point and to optimize its concentration, while stabilizers help
58 adjust pH and color, stop corrosion, and increase hard water stability of formulations (*Dave and*
59 *Ghaly, 2011*).
60   Detergents are continually released into the environment, as a result of their wide industrial
61 applications, contributing to the pollution of rivers, oceans and soil. Surfactants, which are the
62 main components of detergent formulations, are responsible for foaming in rivers and affect the
63 physicochemical properties of soils, causing harm to the organisms that inhabit these ecosystems
64 due to their prolonged presence in the environment (*Drakontis & Amin, 2020; Farias et al.,*
65 *2021; Silva & Sarubbo, 2021)*.
66   Most surfactants in commercial detergents and degreasing formulations are synthesized
67 from petroleum. However, environmental legislation has motivated the development of natural
68 surfactants, such as microbial surfactants, as alternatives to existing products. These
69 biosurfactants are produced from renewable sources and are efficient, non-toxic, biodegradable
70 and stable under extreme environmental conditions *(Farias et al., 2021; Markande et al., 2021)*.
71   Many types of biosurfactants have been produced in recent decades, especially those
72 obtained from bacteria of the genus *Pseudomonas*, which mainly produce glycolipid
73 biosurfactants composed of a hydrophilic head formed by rhamnose molecules and a
74 hydrophobic tail that contains fatty acids *(Bezerra et al., 2018)*. The production of rhamnolipids
75 by *Pseudomonas* has the advantages of obtaining a high-quality surfactant within a short culture
76 time. However, further processing often poses a problem in the production of rhamnolipids,
77 especially due to the low yields *(Płaza & Achal, 2020)*.
78   The development of strategies that enable the application of biosurfactants on an industrial
79 scale is of fundamental importance. Such strategies include the selection of adequate substrates,
80 the determination of optimal culture conditions and the improvement of purification processes
81 *(Santos et al., 2016)*. Moreover, depending on the application purpose, the purification step can
82 be eliminated, which substantially reduces the cost of the process. Thus, the oil industry and
83 environmental applications have become greatest markets for these biomolecules, as such
84 applications have minimal purity specifications *(Jimoh & Lin, 2019)*.
85   Since the cost of production is still a limitation for establishing biosurfactants on the
86 market, they have been blended with various types of cheaper synthetic surfactants. In general,
87 mixed surfactant systems provide superior properties compared to individual surfactants, such as
88 lower interfacial tension *(Shah et al., 2019)*. For example, the aqueous binary system of the
89 Gemini cationic surfactant, ethanediyl-1,3 bis (dodecyl dimethyl ammonium bromide)

90   (represented as 12-3-12) and the bacterial surfactant Surfactin was able to reduce the interfacial
91   tension of petroelum *(Lin et al., 2016)*. *Jian et al. (2011)* described that binary mixtures of
92   synthetic surfactants and plant biosurfactants (saponin) showed more foaming capacity and less
93   interfacial activity than the individual components. Song et al. (2013) showed that a mixture of
94   soporolipids and rhamnolipid with polysorbate-80, sorbet-40 and ethylene glycol butyl was able
95   of dispersing crude oil. Bio-based dispersants have also been formulated by mixing
96   biosurfactants with chemical surfactants, as their synergistic behavior can increase pollutant
97   washing efficiency by increasing the pollutant solubility *(Zhu et al., 2020; Baharuddin et al.,*
98   *2020)*. A bio-based washing agent prepared by mixing 0.3% lipopeptides (an anionic
99   biosurfactant from *Bacillus subtilis* GY19) and 2% Dehydol LS7TH (a nonionic fatty alcohol
100   ethoxylate oleochemical surfactant) removed 92% of 15% (w/w) petroleum hydrocarbons
101   *(Arpornpong et al., 2020)*.
102   Although upstream and downstream processes for the production of biosurfactants have
103   been extensively investigated, studies describing the formulation of novel, efficient products
104   containing biosurfactants are still restrict. Therefore, the aim of this work was to investigate the
105   potential application of biosurfactants produced by bacteria of the genus *Pseudomonas* from
106   previously established substrates and culture conditions in the formulation of a detergent capable
107   of removing heavy oils. The study of a newly formulated washing agent based on the use of
108   microbial surfactants can become the commercial application of these biomolecules more
109   feasible in the near future.
110

Materials & Methods

Microorganisms

113   The bacterial strains *Pseudomonas cepacia* CCT6659 (obtained from the culture bank of the
114   André Tosello Research and Technology Foundation located in the city of Campinas, São Paulo,
115   Brazil) *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* ATCC 10145 and
116   *Pseudomonas aeruginosa* UCP 0992 (obtained from the culture bank of the Environmental
117   Science Research Center (NPCIAMB) of the Catholic University of Pernambuco, Brazil) were
118   used as biosurfactant producers. The cultures were maintained in test tubes with solid nutritive
119   agar slants under refrigeration at 4 °C.
120

Preparation of inoculum

122   For bacterial growth, the CN (nutrient broth) medium was used with the following composition:
123   meat extract (0.1%), yeast extract (0.2%), peptone (0.5%) and sodium chloride (0.5%) at pH 7.0.
124   The growth parameters were temperature of 28 ºC and stirring at 150 rpm for 16 hours until
125   obtaining an optical density (OD) of 0.7 (corresponding to an inoculum of 10^7 CFUs/mL) at 600
126   nm with a concentration of 2.0% (v/v).
127

Production of biosurfactants

129   The culture conditions and production media tested for each microorganism were initially
130   established based on previous experiments carried out at our laboratories. The *P. cepacia*
131   CCT6659, *P. aeruginosa* UCP 0992, *P. aeruginosa* ATCC 9027 and *P. aeruginosa* ATCC
132   10145 strains were then tested for biosurfactant production using the carbon sources and culture
133   conditions described in Table 1.
134   The biosurfactant from *P. cepacia* CCT6659 was produced in a mineral medium containing
135   0.05% KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.01% KCl, 0.001% $FeSO_4.7H_2O$ and 0.

136 2% NaNO$_3$ supplemented with carbon and nitrogen sources, as described by *Soares da Silva et*
137 *al. (2017)*. The biosurfactants from *P. aeruginosa* UCP 0992, *P.aeruginosa* ATCC 9027 and *P.*
138 *aeruginosa* ATCC 10145 were produced in a mineral medium containing 0.1% K$_2$HPO$_4$, 0.1%
139 K$_2$HPO$_4$, 0.02% MgSO$_4$.7H$_2$O , 0.02% CaCl$_2$.H$_2$O and 0.005 % FeCl$_3$.6H$_2$O supplemented with
140 the substrates described in Table 1. Medium surface tensions were between 55-57 mN/m prior to
141 inoculation.

142

Isolation of biosurfactants
144 The most promising media and culture conditions based on the best surface tension values were
145 selected for isolation of the biosurfactants, which were extracted with the addition of ethyl
146 acetate (C$_4$H$_8$O$_2$) to the broth obtained after fermentation (1:1, v/v). The mixture was centrifuged
147 at 5000 rpm for 10 min, stirred vigorously for 15 minutes and allowed to stand until phase
148 separation. The organic phase was removed, and the operation was repeated twice more. The
149 product was concentrated from the combined organic phases, which were evaporated at 40°C in
150 a rotary evaporator. A viscous yellowish product was obtained, which was treated with NaOH
151 and washed with acetone for maximum removal of impurities and dirt. The yield of the isolated
152 biosurfactant was expressed as g/L.

153

154

Determination of surface tension and critical micelle concentration
156 Surface tension was measured in an automatic tensiometer (KSV Sigma 700, Finland) using a
157 Du Noüy ring. For such, the platinum ring was immersed in dilutions of the isolated
158 biosurfactants (0.1 g/L) in distilled water and the force required to pull the ring through the air-
159 liquid interface was recorded. The critical micelle concentration (CMC) of the previously
160 isolated biosurfactants was determined by measuring the surface tension of a water sample
161 during the gradual addition of the surfactant until reaching a constant tension value. The CMC
162 was expressed as mg/L of the isolated surfactants.

163

Determination of emulsification index
165 To determine the emulsification activity of the biosurfactants, samples of cell-free broth (crude
166 biosurfactant) were analyzed based on *Cooper & Goldenberg (1987)*. For such, 3.0 mL of a
167 hydrophobic compound (soybean oil, corn oil or motor oil) and 3.0 mL of the biosurfactant
168 solution were placed in a test tube and the mixture was vortexed for one minute. After 24 hours,
169 the percentage of the emulsion was calculated as follows:
170 E=100 x EP/H (1)
171 in which EP is the height of the emulsified phase and H is the total height of the mixture (both
172 expressed in centimeters). Emulsifying activity prior to inoculation was used as the negative
173 control for each culture medium. All analyses were performed with four experiments.
174

Dispersion of hydrophobic contaminant
176 The ability of the biosurfactants to disperse an oil slick was simulated in the laboratory by
177 contaminating water samples with 5% motor oil. The crude biosurfactants (cell-free broth) were
178 added at proportions of 1:2, 1:8 and 1:25 (vol/vol) of biosurfactant/oil. Negative controls were
179 carried out with distilled water. The results were observed visually and the relation between the
180 required volume of biosurfactant and dimensions of the formed halo was calculated *(Saeki et al.,*
181 *2009)*.

182

Maximization of selected biosurfactant extraction

183
184 Based on the results of the previous experiments involving the determination of surface tension,
185 the emulsification index and oil dispersion, only one biosurfactant (i.e., that produced by *P.*
186 *aeruginosa* ATCC 10145 cultured in mineral medium composed of 5.0% glycerol and 2.0%
187 glucose for 96 h) was selected for the subsequent experiments. Three biosurfactant extraction
188 and isolation processes (described below) were evaluated considering the increase in yield
189 obtained with few downstream steps.
190 Chloroform/methanol system: For isolation of the biosurfactant, the pH of the cell-free broth was
191 initially adjusted to 2.0 with a 6 M solution of HCl. The same volume of chloroform/methanol
192 (2:1, v/v) was added. The mixture was stirred vigorously for 15 minutes and left to stand for
193 phase separation. The organic phase was removed, and the operation was repeated twice more.
194 The product was concentrated from the collected organic phases using a rotary evaporator *(Silva*
195 *et al., 2020)*.
196 Extraction by acid precipitation: A 2N HCl solution was used to acidify the supernatant
197 containing the biosurfactant until reaching pH 2. The mixture was then incubated for 24 hours at
198 4 °C. The formed precipitate was collected by centrifugation at 10000 rpm and 4 °C for 30 min.
199 The precipitate was then subjected to a drying process for 24 hours and then separated for
200 subsequent tests *(Shah et al., 2016)*.
201 Foam fractionation isolation: A simple fractionation system (Figure 1) was used to recover the
202 biosurfactant contained in the foam. In this system, the cell-free broth was fed into the top of the
203 flask with the aid of a peristaltic pump *(Winterburn et al., 2011)*. The foam was collected in
204 another reservoir, forming a concentrated biosurfactant solution after coalescence. Foam
205 fractionation was performed for 1 h to ensure stable foam production conditions and the sample
206 was collected at the end of the process. Foam fractionation performance was measured in terms
207 of enrichment and biosurfactant recovery standards, which are defined in the following
208 equations:

209 $\text{Enrichment} = \dfrac{Cf}{Ci}$

210 $\text{Recovery} = \dfrac{CfVf}{CiVi} \times 100$

211 in which *Cf* is the concentration of biosurfactant in the foamate, *Ci* is the coincident biosurfactant
212 concentration in the feeder, *Vi* is the initial liquid volume and *Vf* is the volume of foamate
213 collected. To compare the yield obtained with this method, biosurfactant concentration in the
214 foamate was determined through chloroform/methanol extraction.

215

Application of biosurfactant in formulation of industrial detergent

216
217 After isolation, the selected biosurfactant was used as the main component of a detergent. To
218 define the most promising formulation, different formulations were initially investigated using
219 natural components. Biodegradable, non-toxic compounds were selected based on previous
220 studies *(Rocha e Silva et al., 2020)* for the formulation of a detergent capable of removing heavy
221 oil.The formulation consisted of a natural organic solvent to maximize the fluidization of the
222 heavy oil, a thickening fatty alcohol to increase the viscosity of the formula, a gum as an
223 emulsion stabilizer and the microbial biosurfactant for the removal the oily fraction during the
224 surface cleaning process. Previously, the formulation components, i.e., the isolated biosurfactant
225 (10%), the natural organic solvent and the thickening fatty alcohol (10%), were tested (30 mL)
226 separately for oil removal to ensure the viability of the formulation containing the biosurfactant,

227 as it is described below using the surface of a glass slide of known mass contaminated with 100
228 μL of heavy oil.
229   Tests were then conducted to determine the best percentage of each component of
230 the formula. The mixing of all components was performed in a mechanical stirrer (Tecnal
231 LTDA, Brazil) at 2000 rpm for 30 min at 80°C. The formation of phases was followed visually
232 over the course of a month. The purpose of quantifying the compounds was to obtain a stable
233 emulsion with the smallest possible amount of each component. All proportions of the
234 compounds are detailed in Table 2. The formulations were prepared by dissolving the solid
235 components and adding the solvent to complete 100% of the total mixture.
236   The efficiency of the formulations was determined based on the stability of the emulsions
237 (no phase separation) and the removal of heavy oil impregnated on different surfaces, as
238 described below. The formulation with the smallest amounts of the components, best stability
239 and greatest oil removal was selected for tests on different surfaces. The selected formulation
240 was subjected to comparative tests with four synthetic commercial detergents of industrial use
241 (identified as commercial detergent A, B, C and D) for cleaning parts, floors and machinery
242 impregnated with heavy oil. Detergent A is formulated with sodium silicates, amines and
243 alcohols (pH 12.0 to 14.0). Detergent B has phosphoric acid and paint strippers (pH 1.0 to 4.0).
244 Detergent C is formulated with ammonium hydroxide and detergent D is a set of hydrogenated
245 (aliphatic and naphthenic) alkaline solvents with high dielectric strength.
246
247 **Assessment of toxicity of industrial detergent against *Artemia salina***
248 The toxicity test was conducted using microcrustacean (*Artemia salina*) larvae as the toxicity
249 indicator with test solutions of the industrial detergent diluted at proportions of 1:5 and 1:10
250 (v/v) prepared in distilled water and used at concentrations of 1 and 2%. Larvae were used within
251 one day of hatching. The tests were conducted in 15-ml penicillin tubes containing 10 shrimp
252 larvae in 10 ml of seawater. The larvae were observed for 24 hours and mortality was calculated
253 *(Meyer et al., 1982)*. Seawater without biosurfactant was used as the control. Each test was run in
254 triplicate.
255
256 **Assessment of phytotoxicity of industrial detergent**
257 Phytotoxicity of the detergent was evaluated in a static assay involving the cabbage species
258 *Brassica oleracea* (var. capitata), with the analysis of seed germination and root growth, based
259 on *Tiquia et al. (1996)*. Test solutions of diluted detergent at proportions of 1:5 and 1:10 (v/v)
260 were prepared in distilled water at concentrations of 1 and 2%. Toxicity was determined in sterile
261 Petri dishes (10 cm) containing Whatman No. 1 filter paper disks. Ten seeds (previously treated
262 with NaClO) were placed symmetrically in the dish, inoculated with 5 ml of the test solution and
263 maintained at 28°C for five days. Distilled water was used as a control. After incubation in the
264 dark, seed germination, root growth ($\geq$ 5 mm) and the germination index (GI) were calculated
265 according to the formulas below:
266 Relative seed germination (%) = (number of seeds germinated in extract / number of seeds
267 germinated in control) x 100
268 Relative root length (%) = (mean root length in extract / mean root length in control) x 100
269 GI = [(% seed germination) x (% root growth)] / 100%.
270 The mean and standard deviation of triplicate samples of each concentration were calculated.
271

272 **Application of detergent in cleaning of surfaces contaminated with heavy oil**
273 The detergent formulated with the isolated biosurfactant was evaluated for heavy oil removal
274 from different types of contaminated surfaces, i.e., smooth surface (glass slide), metallic surface
275 (threaded nuts) and plastic surface (oil storage container). The heavy oil was obtained from a
276 thermoelectric power plant and classified as B1 Special OCB1 fuel oil (PETROBRAS, Brazil).
277 This oil is a complex mixture of hydrocarbons. Its kinematic viscosity at 60 °C is 620 Cst, its
278 flash point 66°C and its density at 20 °C 0.968 g/mL. Part of the surface of a glass slide of
279 known mass was uniformly contaminated with 100 μL of heavy oil. Metal nuts were completely
280 covered with OCB1 oil. Part of the outer surface of a plastic container was contaminated with
281 oil, simulating an overflow of stored petroleum byproducts. The tests were performed statically
282 (pieces immersed in detergent at rest for three minutes). For the storage container, 100 mL of the
283 detergent was manually spread on the surface and the oil was removed with the aid of absorbent
284 material (sponge, paper towel, etc.). In this case, the percentage of removal was determined
285 gravimetrically and visually. For the glass and metal, the specimens were oven dried at 40 °C for
286 30 minutes and the respective weights were recorded. The removal rate was calculated as
287 follows:
288 $$I = 100 \ x \ ((W_c\text{-}W_w))/((W_c\text{-}W_i))$$
289 in which W_c is the weight of the contaminated test specimen, W_w is weight of the test specimen
290 after washing and W_i is the initial weight of the test specimen.
291
292 **Statistical analysis**
293 The data were submitted to statistical analysis using the one-way procedure in Statistica®
294 (version 7.0), followed by linear one-way analysis of variance (ANOVA). All triplicate results
295 were expressed as mean ± standard deviation. Differences were examined using Tukey's post hoc
296 test, with a 95% significance level.
297
298 # Results
299
300 **Properties of biosurfactants**
301 In this work, bacteria capable of producing surfactants with the ability to mobilize and remove
302 medium and heavy oils used at industrial plants were studied. The properties of the
303 biosurfactants were evaluated and the reduction in the surface tension of the medium was the
304 main parameter for the selection of surfactants.
305
306 *Reduction in surface tension*
307 The results obtained for biosurfactants produced by *P. cepacia* CCT 6659 in different media and
308 culture conditions are shown in Figure 2.
309   The culture medium described by *Soares da Silva et al. (2017)* was used as a comparison
310 to the media evaluated in the present study for the same strain, since *P. cepacia* proved to be an
311 excellent biosurfactant producer in the study cited, achieving surface tension around 26 mN/m.
312   The purpose of this initial stage was to study the most suitable culture media for the
313 production of microbial surfactants considering the technical-economic feasibility of large-scale
314 production and with the aim of increasing the yield with few downstream steps. The selection of
315 the most favorable fermentation parameters for the production process was based on the
316 determination of surface tension. For the biosurfactant obtained from the bacterium *P. cepacia* in
317 the culture media analyzed, temperature exerted no significant influence on fermentation,

318   although more satisfactory surface tension results were obtained at 37°C. However, the most
319   promising result was obtained with the highest concentration of glucose (3.0%) at 28 °C, with
320   which surface tension of 31.60 mN/m was reached.
321   Figure 3 shows the results of the analysis of the biosurfactants produced by *P. aeruginosa*
322   UCP 0992.
323   Temperature also did not exert an influence on the fermentation of *P. aeruginosa* UCP
324   0992. Lower concentrations of glucose and sucrose in the culture enabled a greater reduction in
325   surface tension. The use of lower concentrations of substrates translates to an increase in the
326   economy of the process due to the reduction in input costs, which is essential to increasing the
327   market competitiveness of surfactants.
328   Figure 4 shows the surface tension reduction capacity of biosurfactants produced by the
329   strains *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 9027 at temperatures of 28 and 35
330   °C. The mineral medium containing glycerol and glucose as carbon sources at the two
331   temperatures evaluated was the most promising for the production of biosurfactants. As no
332   significant difference was found between temperatures of 28 and 35 °C during the 96 h of
333   culture, 28 °C was considered more viable for obtaining biosurfactants due to the reduction in
334   electricity costs, making the process more feasible.
335   The surface tension results obtained for the four microorganisms analyzed showed that
336   the strains *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 9027 achieved the best
337   performance when cultivated in the medium containing 5% glycerol and 2% glucose carbon
338   sources for 96 hours at 28ºC, reaching excellent surface tension values of around 25.00 and
339   29.00, respectively. Thus, the biosurfactants produced by these two strains were selected for the
340   evaluation of emulsification and dispersion capacity regarding hydrophobic compounds.
341

342   ***Emulsification index***
343   The results of the emulsification activity tests of biosurfactants from *P. aeruginosa* ATCC 10145
344   and *P. aeruginosa* ATCC 9027 cultivated in the medium containing 5% glycerol and 2% glucose
345   for different hydrophobic compounds can be seen in Figure 5.
346   The biosurfactants from both strains exhibited good emulsification capacity for soybean,
347   corn and motor oils, although with specific affinities for the different hydrophobic compounds
348   evaluated. In general, the biosurfactants exhibited good emulsification efficiency for denser oils,
349   especially motor oil, which is often used in industrial engines. The biosurfactant produced by *P.*
350   *aeruginosa* ATCC 10145 emulsified 97.50% of the motor oil. Furthermore, the emulsions had
351   relatively constant indices in relation to other less dense hydrophobic compounds, forming
352   emulsions that remained stable in the long term.
353

354   ***Dispersion capacity***
355   The dispersion capacity of microbial surfactants obtained from the strains *P. aeruginosa* ATCC
356   10145 and *P. aeruginosa* ATCC 9027 cultivated in the medium containing glycerol and glucose
357   is shown in Figure 6.
358   The data demonstrated excellent dispersant activity. All biosurfactant/motor oil ratios
359   tested were promising, especially the biosurfactant from *P. aeruginosa* ATCC 10145, which
360   exhibited dispersion rates of approximately 99.00%. The results indicate excellent interaction
361   between the biosurfactants and oil, even at oil concentrations 25-fold higher than that of the
362   surfactants.
363

364 ***Critical Micelle Concentration***

365 Another important parameter for evaluating the efficiency of a biosurfactant is the CMC, which
366 is the minimum concentration of biosurfactant necessary for the maximum reduction in surface
367 tension. In the present study, the biosurfactants isolated from the *P. aeruginosa* ATCC 10145
368 and *P. aeruginosa* ATCC 9027 strains exhibited excellent surfactant properties, yields and
369 CMCs (Table 3 and Figure 7).
370   The yields are related to surfactants with low purity specifications, as industries that
371 employ heavy oils does not require surfactants with a high degree of purification. Thus,
372 downstream steps, which represent nearly 60% of total production costs, can be eliminated,
373 making the use of biosurfactants economically advantageous *(Santos et al., 2016)*.
374   The data demonstrate the considerable potential of the biosurfactants evaluated for oil
375 removal purposes. From the results considering surface tension, the emulsification index and oil
376 dispersion, *P. aeruginosa* ATCC 10145 was selected as the best biosurfactant producer. Thus,
377 the biosurfactant produced by this bacterial strain was used as one of the main components in the
378 formulation of a biodegradable detergent for application at industrial plants. In the culture
379 medium containing 5.0% glycerol and 2.0% glucose as carbon sources, the biosurfactant from *P.
380 aeruginosa* ATCC 10145 promoted intense foam formation (Figure 8A-B), indicating
381 considerable emulsifying capacity.
382   Based on the results obtained, the biosurfactant produced from the selected strain under its
383 best culture conditions was isolated, as described above. The yield was approximately 12 g/L.
384 An increase in this yield can be achieved by optimizing the production parameters and studying
385 upstream and downstream technologies for this biomolecule. The isolated biosurfactant was able
386 to reduce the surface tension of water from 71 mN/m to 28 mN/m and form a very stable foam
387 even when diluted (Figure 8).
388

389 **Isolation methods of selected biosurfactant**

390 The surface tension and yield of the biosurfactant from *P. aeruginosa* ATCC 10145 with
391 different extraction methods results are described in Table 4. The best reduction in surface
392 tension of the cell-free broth and the liquid was achieved with the foam fractionation process.
393 The best yield was achieved with foam fractionation and extraction using the
394 chloroform/methanol system. Considering the need to obtain a low-cost surfactant with few
395 downstream steps, the use of the broth and coalesced liquid proved to be more promising.
396

397 **Assessment of toxicity of industrial detergent**

398 Ecotoxicological tests using cabbage (*Brassica oleracea*) seeds and microcrustacean (*Artemia
399 salina*) larvae as indicators demonstrated the absence of toxicity of the detergent formulated with
400 the biosurfactant (Table 5). When compared to commercial detergents used in thermoelectric
401 plants, the formulated detergent composed of 20% natural solvent, 2.0% thickening fatty alcohol,
402 0.5% stabilizing gum and 0.5% biosurfactant presented the same oil removal efficiency with no
403 toxicity, which is a considerable advantage over commercial detergents. It is evident, therefore,
404 that the biodetergent offers better working conditions for cleaning workers, ensuring a healthier
405 environment due to the use of a harmless product.
406

407 **Application of industrial detergent in cleaning of surfaces contaminated with**
408 **heavy oil**

409   Since the detergent formulation has several components, they were previously tested separately
410   for oil removal. These controls were carried out to show their effect on the oil removal efficiency.
411   Table 6 shows percentages of oil the removed by the detergent components. It can be observed
412   that the isolated biosurfactant has a similar efficiency when compared to the organic solvent.
413   After testing each component separately, different components concentrations were evaluated.
414   Among the various component concentrations studied, the best formulation was that containing
415   20% natural solvent, 2.0% thickening fatty alcohol, 0.5% stabilizing gum and 0.5%
416   biosurfactant. This combination of compounds formed a stable emulsion over the evaluation time
417   (one month) capable of removing heavy oil (OCB1) from different surfaces in a short time.
418   The detergent formulation was able to remove 100% of the heavy oil from a glass slide, threaded
419   nut and plastic surface (Figures. 9 and 10). The results indicate the benefit of the detergent and
420   that this formulation has potential for future use in the cleaning of parts, machines and equipment
421   impregnated with oils, greases and petroleum byproducts, such as OCB1 oil, commonly used in
422   industrial plants. The green detergent could be a good solution for industrial cleaning, replacing
423   the conventional products and solvents commonly used in such environments.
424   The selected formulation was also subjected to comparative tests with commercial
425   detergents used in industrial plants, achieving very satisfactory results in the removal of OCB1
426   oil from metal surfaces through processes of solubilization and mobilization. Table 7 presents the
427   results of the comparison of commercial detergents and the detergent formulated with the
428   biosurfactant.
429   The data demonstrated the potential of the green detergent for use in the removal of high-
430   density oil, such as OCB1 oil (heavy oil used as fuel for power generation at thermoelectric
431   plants), the high viscosity of which constitutes an obstacle to the action of commercial
432   detergents/mixtures. Although commercial detergent D achieved the same removal rate (100%),
433   one must bear in mind the considerable corrosive and volatile capacity of the product, which can
434   harm the health of operators and limit the durability of metal parts. In contrast, the detergent
435   formulated with the biosurfactant also achieved 100% removal of the OCB1 oil.
436

Discussion

438   Biosurfactant-producing bacteria are found in a wide variety of habitats from aquatic
439   environments (fresh water, seawater and groundwater) to terrestrial environments (soil, sediment
440   and silt). Environmental conditions exert a direct influence on the type of biosurfactants that
441   microorganisms produce *(Decho & Gutierrez, 2017; Nikolova & Gutierrez, 2021)*.
442   Bacteria of the genus *Pseudomonas* are able to produce different kinds of biosurfactants,
443   including glycolipids (rhamnolipids) and lipopeptides. The most widely studied biosurfactant
444   producer is *P. aeruginosa*, which produces rhamnolipids that form stable emulsions with
445   hydrocarbons *(Bollinger et al., 2020; Shahaliyan et al., 2015)*.
446   The production of biosurfactants by microbial species using renewable substrates and
447   optimizing culture conditions (fermentation time, agitation speed, pH of the medium and
448   nutrients) enables obtaining compounds with distinct properties that make these natural
449   surfactants comparable or even superior to their synthetic counterparts (*Silva et al., 2014*).
450   Indeed, the potential of bacteria of the genus *Pseudomonas* combined with the use of different
451   media and fermentation conditions has enabled obtaining extremely promising biosurfactants in
452   terms of surface tension reduction, dispersion and emulsifying capacity, which are fundamental
453   properties in the selection of the biomolecule to compose a formulation with industrial purposes.

454   The reduction in surface or interfacial tension is considered the main parameter for
455   detecting a surface-active compound in a given production medium. This attraction force is the
456   result of the joint interaction between the molecules of liquids. A potent biosurfactant decreases
457   surface tension as its concentration increases in the aqueous medium, enabling greater interaction
458   between immiscible liquids, such as oil and water (*Silva et al., 2014*). According to *Eslami et al.*
459   *(2020)*, a more than 8 mN/m reduction in surface tension is needed to identify a microorganism
460   as a biosurfactant producer. However, rhamnolipids can reduce the surface tension of water from
461   72 to less than 30 mN/m. In the present study, it was possible to obtain a biosurfactant with
462   excellent surface tension reduction capacity through the different media and culture conditions
463   tested with four bacterial strains, achieving values around 26.40 mN/m.
464   The literature offers several studies on biosurfactants produced by species of
465   *Pseudomonas* with similar properties to those obtained in this research. For instance, *Patowary*
466   *et al. (2017)* produced a biosurfactant from the strain *P. aeruginosa* PG1 isolated from soil
467   contaminated with hydrocarbons that reduced the surface tension of the culture medium from
468   51.80 to 29.60 mN/m. *Câmara et al. (2019)* also obtained a biosurfactant from *P. aeruginosa*
469   able to reduce the surface tension of water from 72.00 to 35.26 mN/m. Using the strain *P.*
470   *aeruginosa* NCIM 5514 cultivated in a medium with crude oil as the carbon source, *Varjani &*
471   *Upasani (2019)* produced a biosurfactant able to reduce the surface tension to 29.14 ± 0.05
472   mN/m.
473   Most microbial surfactants are substrate specific, solubilizing or emulsifying different
474   hydrocarbons at different rates. The inability to emulsify some hydrocarbons may be due to the
475   inability of the biosurfactant to stabilize microscopic droplets *(Bouassida et al., 2018)*. In this
476   work, the biosurfactant from *P. aeruginosa* ATCC 10145 exhibited considerable affinity with
477   motor oil, as demonstrated by the emulsification rates higher than 97% obtained for this
478   substrate. In contrast, the vegetable oils tested were not emulsified to the same degree, although
479   50% of these oils were also stably emulsified by the biomolecule. This affinity with motor oil is
480   related to the chemical structure of the biosurfactants evaluated *(Sousa et al., 2014)*, which
481   enables greater intermolecular interaction with the chemical structure of the oil, making these
482   microbial surfactants suitable for applications in industrial environments that generate mineral
483   oil waste *(Almeida et al., 2016)*. *Anaukwu et al. (2020)* described the optimization of
484   biosurfactant production by *P. aeruginosa* to determine the impact of different renewable waste
485   products. The preferred carbon source of the bacterial isolate was sugarcane molasses. The
486   biosurfactant achieved an emulsification index of 96.3% ± 0.75%. In the study by *Bezerra et al.*
487   *(2020)*, the emulsification index of a biosurfactant from *P. aeruginosa* reached 71.0% for
488   vegetable oils.
489   Another important parameter for evaluating the efficiency of a biosurfactant is the CMC,
490   which is the minimum concentration of biosurfactant necessary for the maximum reduction in
491   surface tension. The increase in the concentration of a surfactant in the medium promotes the
492   formation of micelles, which are aggregated amphipathic molecules with the hydrophilic portion
493   positioned outwards and hydrophobic portion positioned inwards, enabling the trapping of oil in
494   the micellar complex and promoting emulsification *(Arsene et al., 2021)*. The CMC is another
495   important parameter to consider in the development of large-scale production processes, as it
496   enables the prediction of efficiency and economic viability *(Santos et al., 2016)*. The CMC found
497   for the biosurfactants from *P. aeruginosa* ATCC 10145 and ATCC 9027 were higher than many
498   values described in the literature for potent bacterial surfactants, such as the biosurfactant
499   produced by *P. aeruginosa* S5, which had a CMC of 96.50 mg/L and reduced surface tension

500   from 72.20 to 29.60 mN/m *(Sun et al., 2019)*. Similar results were also found in the work by
501   *Anaukwu et al. (2020)*, with the surface tension of distilled water reduced from 72.10 mN/m to
502   35.00 ± 0.0 mN/m and a CMC of 60.00 mg/L.
503   Although biosurfactant isolation techniques still need to be improved, the yields obtained
504   for *P. aeruginosa* biosurfactants in this work were satisfactory when compared to those
505   described in the literature for other microbial biosurfactants. The yields obtained are related to
506   surfactants with low purity specifications, as industries that employ heavy oils does not require
507   surfactants with a high degree of purification. Thus, downstream steps, which represent nearly
508   60% of total production costs, can be eliminated, making the use of biosurfactants economically
509   advantageous.
510   An oil dispersant consists of a mixture of various chemicals, but the major
511   constituents are surfactants, which are responsible for solubilization and dispersion. Due to their
512   amphipathic structure, surfactants solubilize oil through the formation of micelles, which
513   disperse in water *(Silva et al., 2014)*. Corexit is one of the most widely used chemical dispersants
514   for oil solubilization. This chemical agent passed initial testing by the US Environmental
515   Protection Agency (EPA) after the government asked to cut its use in half. For the test, the EPA
516   analyzed eight chemicals to determine how oil dispersants affect marine wildlife. All products
517   had negative effects despite the favorable dispersant performance. The EPA still permits the use
518   of Corexit but stresses the need to find a more environmentally friendly alternative (Silva et al.,
519   2014). In this scenario, biosurfactants constitute a promising alternative to replace chemical
520   dispersants in formulations. The dispersion results obtained in the present study for
521   biosurfactants from the strains *P. aeruginosa* ATCC 10145 and ATCC 9027 were above 70%
522   regardless of the type of oil and dilution tested and reached rates of up to 95%, demonstrating the
523   capacity of these biomolecules as dispersing agents. Other biosurfactants produced by species of
524   *Pseudomonas* have proven to be highly efficient dispersants of petroleum byproducts. *Soares da*
525   *Silva et al. (2017)* demonstrated that the biosurfactant from *P. cepacia* dispersed oil (81%),
526   indicating the potential of the biosurfactant for application in the control of oil spills. *Sun et al.*
527   *(2019)* proved that the glycolipid biosurfactant produced by a strain of *P. aeruginosa* was able to
528   disperse approximately 74% of petroleum byproduct in water. A new green detergent similar to
529   the formulation described in this work, composed by 1.0% of the biosurfactant from *Starmerella*
530   *bombicola* ATCC 22214, 0.4% of hydroxyethyl cellulose, 1.0% of EDTA and 0.2% of potassium
531   sorbate as preservative was tested in the remediation of soils contaminated with hydrocarbons
532   *(Silva et al., 2021)*. The formulation showed effectiveness in removing motor oil from
533   contaminated sandy soil (80.0%) and beach sand (65.0%) under static conditions. Although the
534   conditions applied were totally different form the experiments carried out in our research, the
535   static condition can be compared to the immersion tests carried out for heavy oil removal and
536   shows, once more, the feasibility of detergent formulations based on biosurfactants.
537   Studies have reported that some forms of extraction and isolation are more adequate for
538   specific biosurfactants. In the present study, three forms of isolation were evaluated for the
539   biosurfactant from *P. aeruginosa* ATCC 10145 to select the most economically viable extraction
540   method capable of obtaining the highest concentration of biosurfactant for application in the
541   formulation of the detergent. The most promising isolation method was foam fractionation.
542   Several studies in the literature report the characteristics of the foaming method of biosurfactants
543   in fermentation broths and the use of foaming for product recovery. *Beuker et al. (2016)*
544   evaluated the efficacy of foam fractionation in rhamnolipid production. The foam fractionation
545   process was easily manageable in an enriched bioreactor and the biosurfactants were highly

546 concentrated in the foam during the culture process, with an increase in rhamnolipid recovery of
547 up to 97%. *Bages-Estopa et al. (2018)* used the foaming method for trehalolipid biosurfactants in
548 fermentation broths. Trehalolipids were produced by the bacterium *Rhodococcus* sp. PML026
549 with hexadecane as carbon source. Hexadecane suppresses foaming during fermentation, which
550 is beneficial during the growth and production phases. In the study, the hexadecane substrate was
551 exhausted at the end of the fermentation, enabling the formation of foam and the separation of
552 the product by foam fractionation, with a total trehalolipid recovery rate of 23 to 58%. *Blesken et*
553 *al. (2020)* proposed this method as a pre-purification step. Foam fractionation was coupled to the
554 bioreactor operation using an external fractionation column to decouple the production of a
555 rhamnolipid by *P. putida* KT2440. The technique enabled continuous separation of the
556 surfactant, which is particularly suitable for scale-up. Surfactant concentrations of 7.50 g/L were
557 obtained in the fractionated foam. These studies demonstrate a strategic, low cost, eco-friendly
558 technology for separating biosurfactants from emulsified fermentation broths.
559   The absence of toxicity is of fundamental importance for the application of a product in
560 industrial sectors. Detergents and surfactants are among the most widely used chemicals in
561 industries. The toxic effect of a detergent depends on its mode of action, the toxicity of the active
562 ingredient (surfactant) and the response of organisms. Short-term ecotoxicity bioassays are
563 analytical methods that enable the assessment of bioavailability and the toxicity of chemical
564 substances by showing acute effects on the survival or mobility of test organisms. The toxicity
565 tests conducted in the present study revealed the non-toxic nature of the detergent formulated
566 with the *P. aeruginosa* biosurfactant ATCC1045, demonstrating the viability of this novel
567 product in the market. The detergent formulation produced in this work also achieved better
568 results in comparison to microbial biosurfactants described in the literature *(Ostendorf et al.,*
569 *2019; Rocha e Silva et al., 2014)*.
570   The literature describes several studies conducted to determine the toxicity of chemical
571 detergents on test organisms, but little is known about the toxic effects of biobased detergents,
572 which are considered to be environmentally friendly. *Uc-Peraza & Delgado-Blas (2015)* studied
573 the toxicity of three commercial detergent formulations (ROMA®, FOCA® and BLANCA
574 NIEVES®) to determine the median lethal concentration (LC_{50}). FOCA® was the most toxic
575 detergent, followed by BLANCA NIEVES ® and ROMA ®. According to the authors,the
576 difference in toxicity among the three detergents can be related to the concentrations of the
577 anionic surfactants or to other ingredients such as sodium silicate, enzymes, sodium
578 tripolyphosphate, bleaches and perfumes. In acute toxicity tests with fish, *Baharuddin et al.*
579 *(2020)* found that a dispersant based on water and ionic liquids (ILs) [1-butyl-3-
580 methylimidazolium lauroylsarcosinate, 1,1'-(1,4-butanediyl)bis (1-H-pyrrolidinium)
581 dodecylbenzenesulfonate, tetrabutylammonium citrate, tetrabutylammonium polyphosphate and
582 tetrabutylammonium ethoxylate oleyl ether glycolate] was practically non-toxic and exhibited
583 excellent biodegradability throughout the test period. This novel surfactant was considered an oil
584 facilitator and environmentally safe for use in oil spill remediation process and could effectively
585 replace toxic chemical dispersants. *Rocha and Silva et al. (2020)* developed a sustainable plant-
586 based biodetergent composed of a natural solvent (cotton seed oil), plant surfactant agent
587 (saponin) and two natural stabilizers (carboxymethylcellulose and glycerin), which exhibited
588 stability, efficiency (removing 100% of heavy oil from metallic surfaces) and the absence of
589 toxicity. According to the authors, the application of the novel product would reduce both
590 environmental impact and the risk posed to worker health related to toxic cleaning products.
591 *Arpornpong et al. (2020)* investigated improved washing technology to treat drill cuttings from

592   oil exploration and production sites with high concentrations of total petroleum hydrocarbons
593   (TPHs). For such, a biologically based washing agent was formulated with a lipopeptide
594   biosurfactant (in foam or cell-free broth), Dehydol LS7TH (fatty alcohol ethoxylate 7EO – an
595   oleochemical surfactant) and butanol (as a lipophilic binder) in water. Due to the synergistic
596   behavior between the anionic lipopeptides and the nonionic Dehydol LS7TH, the formula
597   removed 92% of the TPHs from the drill cuttings when applied in a test jar. In a study conducted
598   by *Helmy et al. (2020)*, the application of biosurfactants in the formulation of a washing
599   detergent was investigated and compared to that of a standard commercial detergent. The
600   formulation comprising a mixture of a rhamnolipid, sodium tripolyphosphate as a builder and
601   sodium sulphate as a filler was applied to wash stained cotton fabric. The results showed that the
602   biosurfactant and its formulations are a promising substitute for synthetic counterparts, as for the
603   results obtained in this work.
604   Therefore, biobased detergents are efficient and sustainable. In addition to promoting
605   cleaning, these products are not toxic and have greater stability compared to conventional
606   products. Biodetergent production will not generate any hazardous or environmentally harmful
607   waste and does not rely on highly toxic raw materials. Thus, the use of green detergents is no
608   longer merely a promise for industrial fields; it is a real possibility and an emerging necessity.
609
610

611   Conclusions

612   Advances in sustainable technologies have increasingly driven the search for natural
613   biodegradable compounds that can achieve direct and indirect reductions in impacts on the
614   environment, such as green formulations based on microbial surfactants. The biosurfactant from
615   *P. aeruginosa* ATCC 10145 demonstrated viability for the formulation of a non-toxic detergent,
616   with a high capacity to remove heavy oil impregnated on different surfaces. Thus, the present
617   results demonstrate an innovative product that is superior in efficiency compared to chemical
618   detergents currently on the market and is capable of reducing the environmental impacts arising
619   from contamination by heavy oils. Since this is a laboratory study, viability for industrial
620   application needs large-scale studies. Other parameters, such as aroma, color and long-term
621   stability, also need to be considered. A future, more in-depth investigation with the on-site
622   application of this novel product could indicate more applications in common industrial
623   activities.

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628

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Media and culture conditions evaluated for production of biosurfactants by strains of *Pseudomonas*.

Microorganisms	Production media and fermentation conditions	
	Carbon sources	Cultivation parameters
Pseudomonas cepacia CCT6659	2.0% canola frying oil and 3.0% corn steep liquor *(Soares da Silva et al., 2013)*	pH 7.0; 250 rpm; 60 h; 28 and 37°C
	5.0% glycerol and 2.0% glucose	pH 7.0; 200 rpm; 96 h; 28 and 37°C
	1.5% glucose	
	2.0% glucose	
	3.0% glucose	
Pseudomonas aeruginosa UCP0992	1.5% glucose	pH 7.0; 200 rpm; 96 h; 28 and 37°C
	2.0% glucose	
	3.0% glucose	
	2.0% sucrose	
	3.0% sucrose	
Pseudomonas aeruginosa ATCC 9027 and *Pseudomonas aeruginosa* ATCC 10145	5.0% glycerol and 2.0% glucose	pH 7.0; 200 rpm; 96 h; 28 and 35°C
	1.0% N-hexadecane and 1.0% glucose	
	2.0% sugar cane molasses and 3.0% corn steep liquor	

Compounds evaluated for formulation of industrial detergent containing biosurfactant.

Components	Quantities (%)
Natural organic solvent (v/v)	20.0 and 30.0
Isolated biosurfactant (w/v)	0.5 and 1.0
Thickener fatty alcohol (w/v)	0.5, 1.5, 2.0, 2.5 and 3.0
Stabilizing gum (w/v)	0.4, 0.5 and 0.6

Properties of biosurfactants from *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 9027 cultivated in medium composed of 5.0% glycerol and 2.0% glucose for 96 h at 28°C.

Microorganisms	Surface tension (mN/m)	CMC (mg/L)	Yield (g/L)
Pseudomonas aeruginosa ATCC 9027	28.00 ± 0.37	20.00	1.20 ± 0.08
Pseudomonas aeruginosa ATCC 10145	26.40 ± 0.32	60.00	11.97 ± 0.29

Surface tension and yield of biosurfactant from *P. aeruginosa* ATCC 10145 cultivated in medium composed of 5.0% glycerol and 2.0% glucose with different extraction methods.

Biosurfactant and extraction methods	Surface tension (mN/m)	Yield (g/L)
Cell-free broth (crude biosurfactant)	24.06 ± 0.22	-
Biosurfactant isolated with chloroform/methanol	30.60 ± 0.13	1.82 ± 0.02
Biosurfactant isolated by acid precipitation	29.43 ± 0.15	0.44 ± 0.00
Biosurfactant isolated by foam fractionation	26.50 ± 0.22	11.45 ± 0.21%*

1   * % v/v

Toxicity tests of detergent formulated with 20% natural solvent, 2.0% thickening fatty alcohol, 0.5% stabilizing gum and 0.5% biosurfactant from *P. aeruginosa* ATCC 10145.

Detergent/water ratio (v/v)	Mortality of *Artemia salina* larvae (%)	*Brassica oleracea* seed germination (%)
1/5	10.00 ± 0.01	100.00 ± 0.01
1/10	No mortality	100.00 ± 0.00

Removal of heavy oil (OCB1) from glass slide by the components of detergent formulated with biosurfactant from *P. aeruginosa* ATCC 10145 cultivated in 5.0% glycerol and 2.0% glucose for 96 h at 28°C.

Formulation components	Oil removal
Natural organic solvent	40±0.51%
Isolated biosurfactant	40±0.37%
Thickener fatty alcohol	25±0.13%

Removal of heavy oil from metal surfaces by commercial detergents and detergent formulated with biosurfactant from *P. aeruginosa* ATCC10145 cultivated in 5.0% glycerol and 2.0% glucose for 96h at 28°C.

Detergents	Removal of OCB1 heavy oil (%)
Water (control)	2.10 ± 0.05
Commercial detergent A	17.30 ± 0.06
Commercial detergent B	10.20 ± 0.10
Commercial detergent C	42.50 ± 0.15
Commercial detergent D	100.00 ± 0.05
Detergent formulated with biosurfactant	100.00 ± 0.01

3 * Commercial detergent A: formulated with sodium silicates, amines and alcohols; Commercial
4 detergent B: formulated with phosphoric acid and paint strippers; Commercial detergent C:
5 formulated with ammonium hydroxide; Commercial detergent D formulated with set of
6 hydrogenated (aliphatic and naphthenic) alkaline solvents with high dielectric strength.

Figure 1

Illustration of foam fractionation method. Air inlet and foam formation from *P. aeruginosa* ATCC 10145 cell-free broth (1); foam transport to receiving flask (2); foam coalescence and air outlet (3).
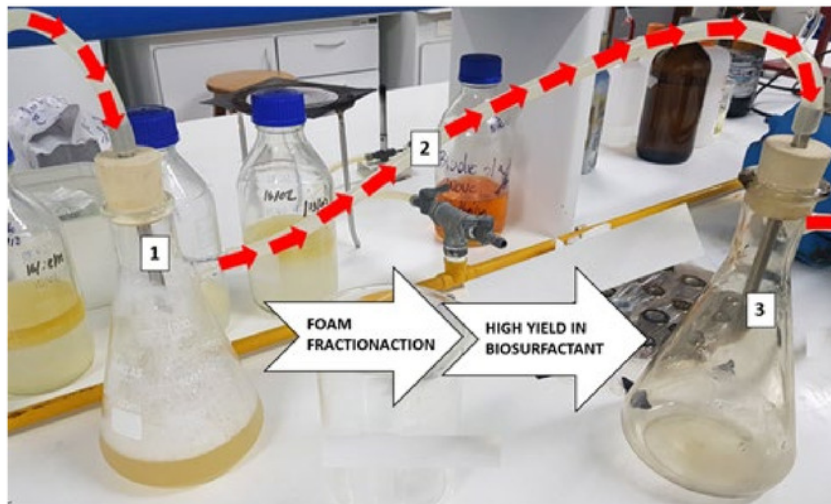
Figure 2

Surface tensions after culture of *P. cepacia* CCT6659 (60 h, 250 rpm) at 28 and 37°C (Soares da Silva et al., 2013) and at 28 and 37°C (96 h, 200 rpm) in glycerol and glucose (A) and in glucose (B).

Data presented are the average of triplicate experiments and error bars indicate standard deviation around the mean (***0.2- 0.5; **0.6 – 1.4).
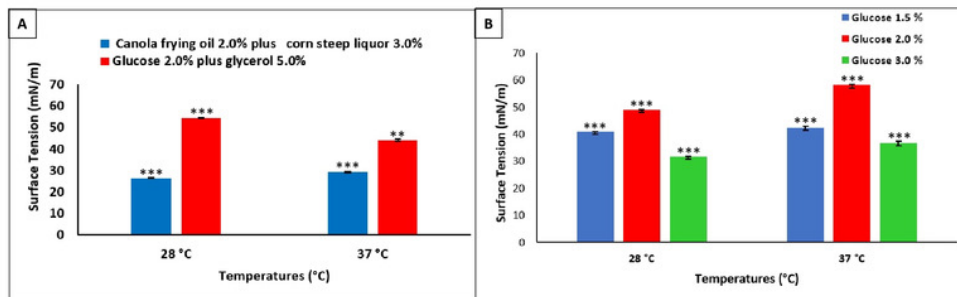
Figure 3

Surface tensions obtained after culture of *P. aeruginosa* UCP 0992. Fermentations performed for 96 hours at 200 rpm at 28 and 37°C in media containing sucrose (A) and glucose (B).

Data presented are the average of triplicate experiments and error bars indicate standard deviation around the mean (***0.2- 0.5).
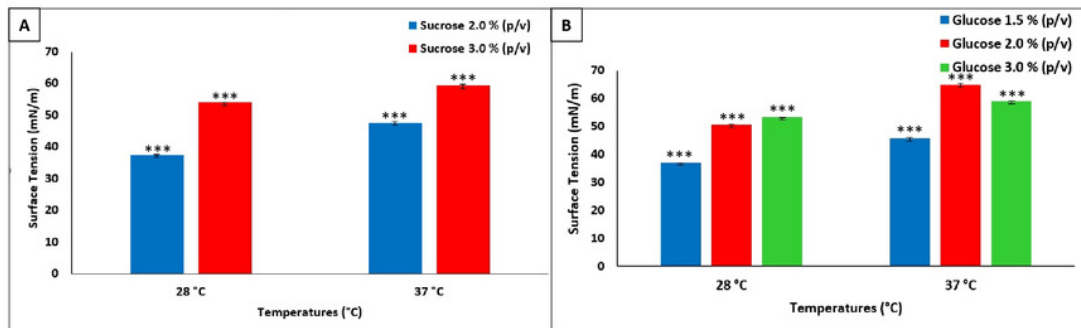
Figure 4

Surface tensions after culture of *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 9027 in glycerol plus glucose, hexadecane plus glucose and molasses plus corn steep liquor at 28°C (A) and 35°C (B).

Data presented are the average of triplicate experiments and error bars indicate standard deviation around the mean (***0.2- 0.5; **0.6 – 1.4).
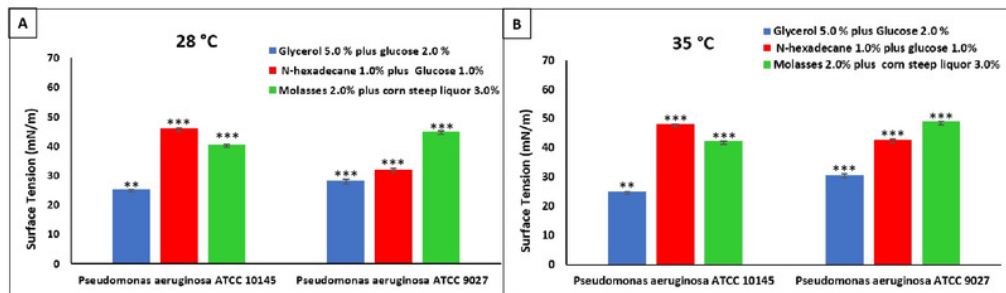
Figure 5

Emulsification capacity of biosurfactants from *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 9027 in medium composed of 5.0% glycerol and 2.0% glucose for 96 h at 28°C with different oils.

Data presented are the average of triplicate experiments and error bars indicate standard deviation around the mean (***0.2- 0.5; **0.6 – 1.4; * 1.5 – 2.0).
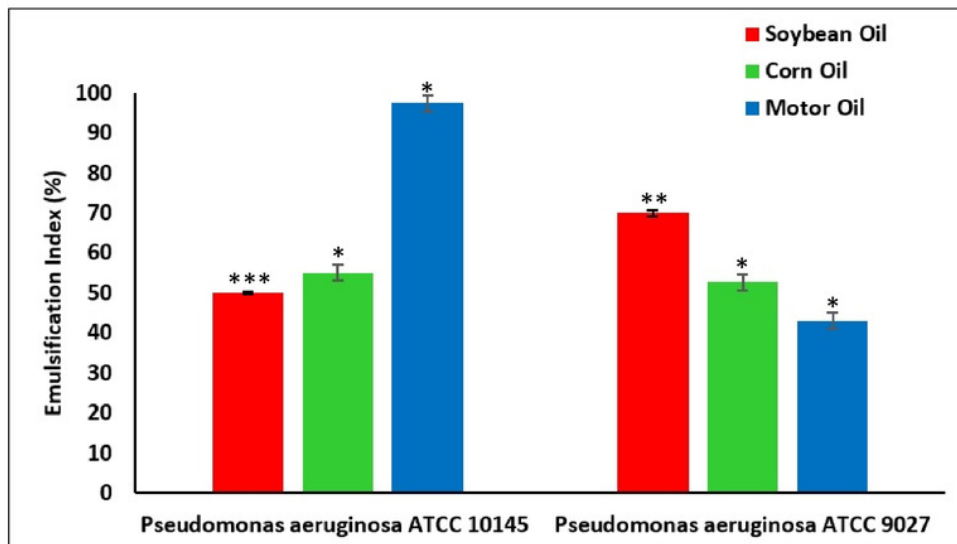
Figure 6

Dispersion of motor oil by biosurfactants from *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 9027 cultivated in 5.0% glycerol and 2.0% glucose for 96 h at 28°C.

Data presented are the average of triplicate experiments and error bars indicate standard deviation around the mean (***0.2- 0.5; **0.6 – 1.4).
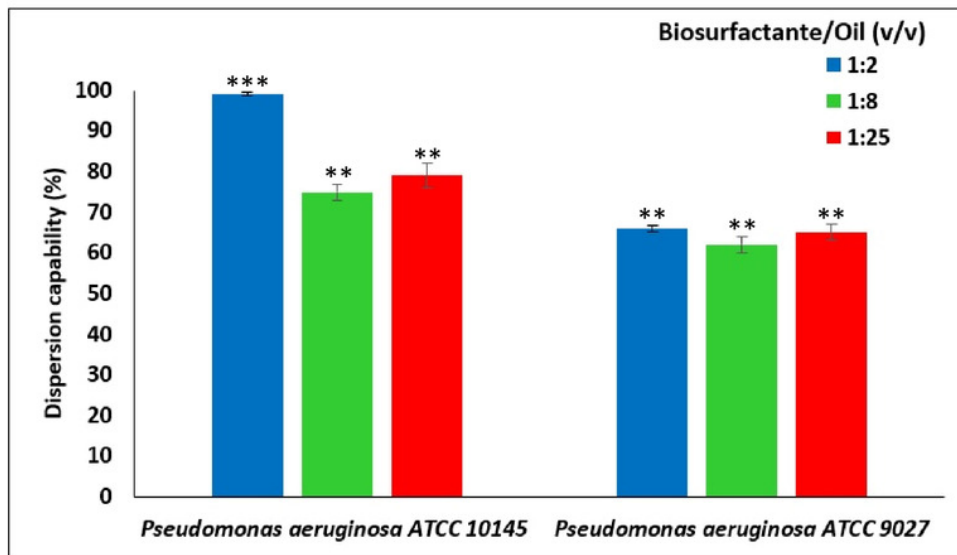
Figure 7

Critical micelle concentration plots of the biosurfactants produced from (A) *P. aeruginosa* ATCC 10145 and (B) *P. aeruginosa* ATCC 9027 cultivated in 5.0% glycerol and 2.0% glucose for 96 h at 28°C.
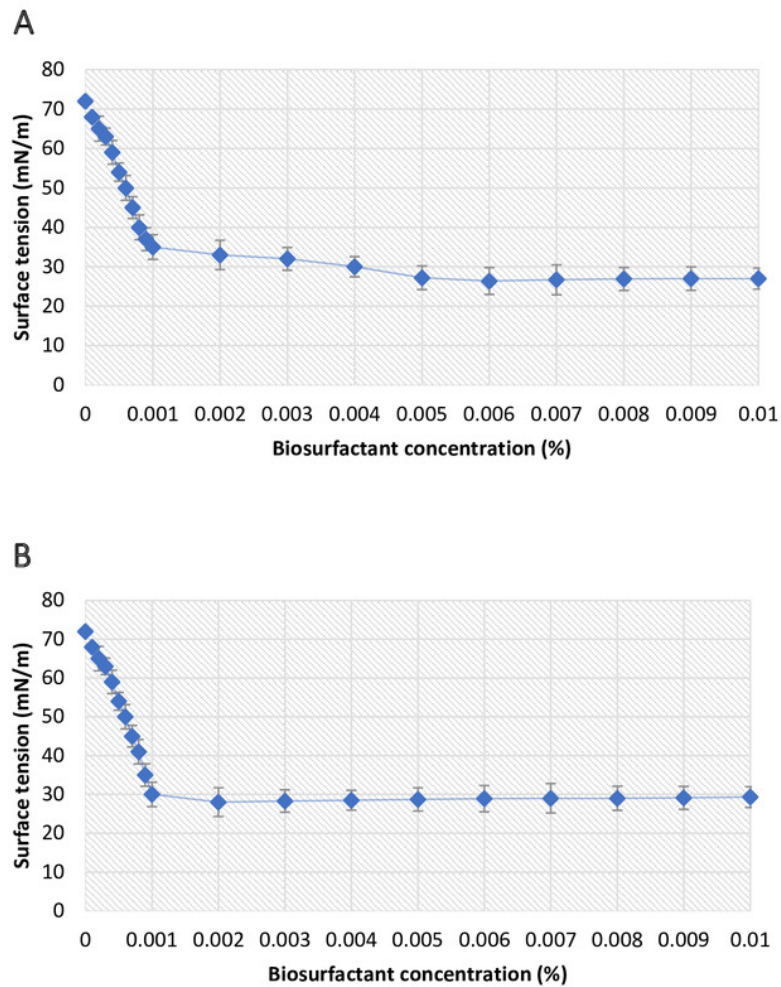
Figure 8

Foam formation promoted by biosurfactant from *P. aeruginosa* ATCC 10145 cultivated in 5.0% glycerol and 2.0% glucose for 96 h at 28ºC. Crude biosurfactant (A and B) and isolated biosurfactant (C).
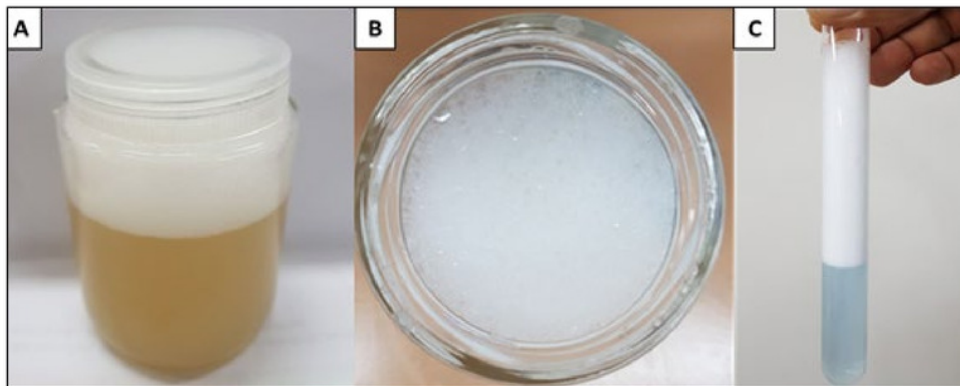
Figure 9

Removal of heavy oil (OCB1) from contaminated surface by industrial detergent formulated with the biosurfactant from *P. aeruginosa* ATCC 10145 cultivated in 5.0% glycerol and 2.0% glucose.

(A) Heavy oil impregnated glass slide for detergent immersion. (B) Oil-impregnated slide immersed in detergent. (C) Immersion in distilled water for complete removal of destabilized oil from glass surface. (D) Complete oil removal.
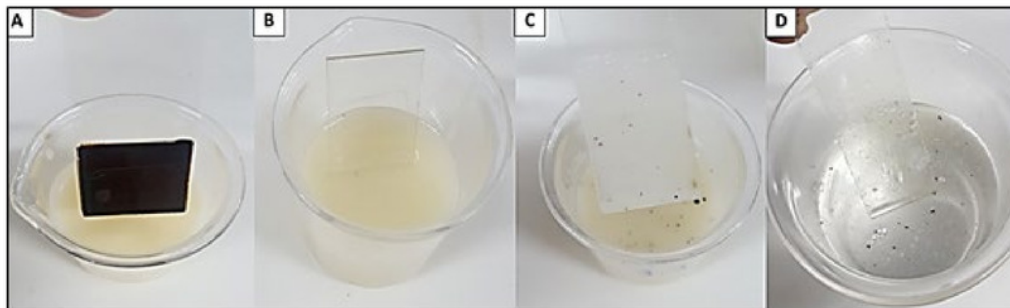
Figure 10

Removal of heavy oil (OCB1) from metallic and plastic surfaces by industrial detergent formulated with biosurfactant from *P. aeruginosa* ATCC 10145 cultivated in 5.0% glycerol and 2.0% glucose.

I - Metallic surface. (A) Threaded nuts impregnated with heavy oil OCB1 for immersion in detergent threaded. (B) Nuts impregnated with oil immersed in detergent. (C) Immersion in distilled water for complete removal of destabilized oil from metal surface. (D) Complete oil removal. II - Plastic surface. (A) OCB1 oil-impregnated plastic storage container for detergent application. (B) Application of detergent to oil-impregnated surface. (C) Complete oil removal.