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Innovation in the production of ecological biodispersants: co-cultivation of *Serratia marcescens* and *Tetradesmus obliquus*

Inovação na produção de biodispersantes ecológicos: co-cultivo de *Serratia marcescens* e *Tetradesmus obliquus*

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ABSTRACT

Mixed fermentations with bacteria and microalgae have been successfully used to increase biomass and metabolites production. However, this strategy has not yet been explored to produce biodispersants—biomolecules with potential for use in the bioremediation of petroderivatives. Therefore, we investigated the production of biosurfactants by co-cultivation of *Serratia marcescens* and *Tetradesmus obliquus* and its application as a biodispersant. The biomolecule was isolated by acid precipitation and subjected to preliminary characterization, stability and phytotoxicity tests and application in removing burnt engine oil from mollusk shells. When cultivated alone, *S. marcescens* presented surface tension of 27.4 mN/m and oil displacement area of 34.54 cm^2, and when cultivated with *T. obliquus*, presented 26.6 mN/m and 50.24 cm^2, respectively. Furthermore, excellent results of interfacial tension (1.0 mN/m) and emulsification index (96%) were verified in the mixed culture. The biosurfactant yield was 1.75 g/L, and it presented an anionic and lipopeptide nature, as well as stability at alkaline pH and in a wide range of temperature and salinity. In addition, it proved to be non-toxic against cucumber (*Cucumis sativus*) and lettuce (*Lactuca sativa*) seeds and showed 100% efficiency in washing mollusk shells impregnated with burnt engine oil. Thus, the co-cultivation of *S. marcescens* and *T. obliquus* represents an innovative and sustainable technology for biodispersant production with a view to application in the bioremediation of environments contaminated with petroleum derivatives.

Keywords: biosurfactant; co-culture; bacterium; microalgae; petroderivative bioremediation.

RESUMO

Fermentações mistas com bactérias e microalgas vêm sendo usadas com sucesso para aumentar a produção de biomassa e metabólitos. Entretanto, essa estratégia ainda não foi explorada para produzir biodispersantes – biomoléculas com potencial de utilização na biorremediação de petroderivados. Diante disso, investigamos a produção de biossurfactante por meio do co-cultivo de *Serratia marcescens* e *Tetradesmus obliquus* e sua aplicação como biodispersante. A biomolécula foi isolada por precipitação ácida e submetida a caracterização preliminar, testes de estabilidade e fitotoxicidade e aplicação na remoção de óleo de motor queimado de conchas de moluscos. Quando cultivada sozinha, a *S. marcescens apresentou* tensão superficial de 27,4 mN/m e área de deslocamento de óleo de 34,54 cm^2, e quando cultivada com *T. obliquus*, apresentou 26,6 mN/m e 50,24 cm^2, respectivamente. Além disso, foram verificados excelentes resultados de tensão interfacial (1,0 mN/m) e índice de emulsificação (96%) no cultivo misto. O rendimento do biossurfactante foi 1,75 g/L, e apresentou natureza aniônica e lipopeptídica, bem como estabilidade em pH alcalino e em ampla faixa de temperatura e salinidade. Além disso, demonstrou ser atóxico frente a sementes de pepino (*Cucumis sativus*) e alface (*Lactuca sativa*) e mostrou 100% de eficiência na lavagem de conchas de moluscos impregnadas com óleo de motor queimado. Assim, o co-cultivo de *S. marcescens* e *T. obliquus* representa uma tecnologia inovadora e sustentável para produção de biodispersante com vistas à aplicação em biorremediação de ambientes contaminados com petroderivados.

Palavras-chave: biossurfactante; co-cultivo; bactéria; microalga; biorremediação de petroderivados.

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Introduction

Oil spills in the oceans have been occurring for many years, contaminating marine ecosystems and causing environmental problems, as they affect their ecological structure, resulting in major losses to aquatic biodiversity (Little et al., 2021). Petroleum and its derivatives are hydrophobic compounds, which hinder the processes of respiration and photosynthesis, interfering with the food chain (Huang et al., 2024). In addition, hydrocarbons negatively affect the structural and functional properties of cell membranes in living organisms, posing a threat to health (Chen et al., 2021; Roy et al., 2024).

One of the most widely used methods in recent decades for the removal of petroleum derivatives during spills is chemical dispersants derived from petroleum (Selva Filho et al., 2023). However, many studies point to the toxic effect of these compounds on aquatic biota and microbial functions (Farahani and Zheng, 2022; Selva Filho et al., 2023). Consequently, their use is becoming increasingly restricted, generating a growing demand for environmentally friendly alternatives with dispersion capacity, with biosurfactants being a promising alternative (Santos et al., 2021; Atakpa et al., 2023; Selva Filho et al., 2023).

However, the high production cost is still a limiting factor for producing biosurfactants on an industrial scale for application in bioremediation (Santos et al., 2016; Selva Filho et al., 2023). In turn, co-cultivation has been an emerging alternative to improve the production of microbial metabolites, since it makes the process less expensive and competitive for industrial purposes (Alves et al., 2019; Araújo et al., 2019). The co-cultivation method provides the most effective use of nutrients due to the beneficial interaction between species, requires no genetic manipulation techniques and expensive reagents, is environmentally friendly, and reduces cultivation time, making the process less expensive (Kiani et al., 2023). In addition, co-cultivation provides a rich metabolic network, which can overcome the limitations of a single microbial species in bioremediation processes, thus ensuring complete biodegradation (Alisi et al., 2009).

There are reports in the literature of co-cultivation using microalgae for biodiesel production (Zhu et al., 2019) and between bacteria for biosurfactant production (Hamza et al., 2018; Alves et al., 2019). Other studies have reported co-cultivation between bacteria and yeast, and between bacteria and microalgae for lipid production (Xu et al., 2018; Karim et al., 2023), as well as between filamentous fungus and bacteria for crude oil biodegradation (Atakpa et al., 2023).

Co-cultivation between bacteria and microalgae stands out for increasing the production of biomass and/or metabolites due to the symbiosis between microorganisms since photosynthetic microalgae release oxygen that is used by aerobic bacterial metabolism. In turn, heterotrophic bacteria oxidize organic carbon (using O_2 from microalgae) and produce CO_2 that is consumed by microalgae as an autotrophic carbon source (Crespo et al., 2023; Kiani et al., 2023). However, there are no studies in the literature on the production of biosurfactants by co-cultivation of bacteria with microalgae.

In this context, *Serratia marcescens* and *Tetradesmus obliquus* were chosen to compose a mixed fermentation, since previous studies have demonstrated their abilities in the production of biosurfactants and greater efficiency in the use of nutrients and growth when co-cultivated, respectively (Araújo et al., 2019; Santos et al., 2021; Zhao et al., 2024). Therefore, the present research aimed to investigate the hypothesis that co-cultivation between the bacterium *S. marcescens* UCP 1591 and the microalgae *T. obliquus* A5F 5402 can improve biosurfactant production and be an innovative and promising process for industrial purposes.

Materials and Methods

Microorganisms and culture media

The bacteria *S. marcescens* UCP 1591 were obtained from the Culture Collection of the Catholic University of Pernambuco (Recife, Pernambuco, Brazil). The microalgae *T. obliquus* A5F 5402 were provided by the Biotechnology Center of the Federal Rural University of Pernambuco (Recife, Pernambuco, Brazil). *T. obliquus* was cultivated in BG-11 medium ($NaNO_3$, K_2HPO_4, $MgSO_47H_2O$, $CaCl_22H_2O$, $C_6H_8O_7$, $(NH_4)_5[Fe(C_6H_4O_7)_2]$, EDTA, Na_2CO_3 and trace metals) (Stanier et al., 1971). The cultivation of *S. marcescens* and the co-cultivation of both microorganisms were carried out in BG-11 medium, supplemented with 5% wheat bran and 5% waste frying soybean oil (WFSO) (Santos et al., 2021).

Production of biosurfactants in monoculture and co-culture

The microorganisms were grown in monoculture and co-culture in 2 L Erlenmeyer flasks containing 1 L of the respective culture medium. The initial cell concentration of the microalgae was 50 mg/L, which represents an inoculation ratio of 1:0.75 (bacteria-algae) in co-cultivation. Fermentation was carried out at 28°C, under continuous light intensity of 45, standard deviation (±) 5 µmol photons $m^{-2}{\cdot}s^{-1}$ and constant aeration with an air pump, for 48 h (Leong et al., 2020). After this time, the cultures were centrifuged for 15 min at 5000 rpm, and the cell-free metabolic liquids obtained were used to determine the surface tension (ST) and oil displacement area (ODA).

Determination of surface tension and oil displacement area

Biosurfactant production was verified by determining the ST of the cell-free metabolic liquids, using the Du Noüy ring method (Kuyukina et al., 2001), at room temperature (approximately 28°C). The ST of distilled water was also measured and used as a control. The dispersion property was investigated by the oil dispersion test, according to Silva et al. (2014), using burnt engine oil as a hydrophobic compound. The ODA was determined and expressed in cm^2. Distilled water and commercial detergent were used as controls. Based on the ST and ODA results, the best condition for obtaining biosurfactants with dispersing properties was selected and used for the analyses described below.

Determination of interfacial tension and emulsification index

Interfacial tension and emulsification index (EI_{24}) were determined using cell-free metabolic liquids, according to Santos et al. (2021).

Biosurfactant extraction

The extraction of the produced biosurfactant was performed by acid precipitation, adjusting the pH of the cell-free metabolic liquid to 2, by adding a 2 M HCl solution, and then leaving it to stand for 24 h at 4°C (Santos et al., 2021). Subsequently, the precipitated biosurfactant was separated from the supernatant by centrifugation (5000 rpm for 15 min at 5°C), frozen, lyophilized by Advantage Plus EL-85 lyophilizer (SP Scientific, USA), and kept in a desiccator until constant weight. The dry weight was determined by gravimetry and the yield was measured in g/L (Araújo et al., 2019).

Preliminary characterization of the biosurfactant

The ionic charge of the crude biosurfactant was determined using the Zeta-Meter 3.0+ system, in a ZM3-DG potentiometer (Direct Imaging, Zeta Meter, Inc., USA) (Santos et al., 2021). In addition, its biochemical composition was determined by quantification of total proteins and enzymatic glucose (In Vitro Diagnóstica Ltda; Itabira-MG, Brazil). The total lipid content was obtained after extraction with chloroform and methanol, according to the methodology of Manocha et al. (1980). The functional groups of the biosurfactant were identified by Fourier transform infrared spectroscopy (FTIR), in the wavelength range of 500–4000 cm^{-1}.

Biosurfactant stability test

The stability of the produced biosurfactant was investigated using the cell-free metabolic liquid. It was separated into small volumes, which were individually subjected to different temperatures (0, 10, 50, 70, and 100°C), adjusted to different pH values (2, 4, 6, 8, 10, and 12) and sodium chloride (NaCl) concentrations (5, 10, 15, 20, 25, and 30%) (Montero-Rodríguez et al., 2015; Santos et al., 2021).

Biosurfactant phytotoxicity test

The phytotoxicity of the produced biosurfactant was investigated according to the methodology of Tiquia et al. (1996), using cucumber (*Cucumis sativus*) and lettuce (*Lactuca sativa*) seeds and different biosurfactant concentrations (0.1, 0.5, and 1%).

Biosurfactant application in the removal of petroleum derivatives from mollusk shells

Mollusk shells were collected from Piedade Beach (Pernambuco, Brazil) and transported to the laboratory for testing. First, the mollusk shells were washed with distilled water and dried at room temperature. They were then weighed and contaminated with burnt engine oil, and the weight was rechecked. Subsequently, the shells were placed in a Falcon tube containing 10 mL of biosurfactant solution and shaken for 2 min. After washing, the mollusk shells were dried in an oven at 60°C and weighed. Oil removal was calculated gravimetrically from the amount of oil remaining in the shells washed with biosurfactant (Santos et al., 2021).

Statistical analysis

All results were subjected to the homoscedasticity test (Levene) and the normality test (Shapiro-Wilk). Analysis of variance (ANOVA) was applied to normal data, and subsequently the Tukey test to detect significant differences. For data that were not normal, the Kruskal-Wallis test was applied, followed by the Dunn test adjusted by Bonferroni. The Mann-Whitney test was also used to evaluate the variance of ST at acidic (2–6) and alkaline (8–12) pH, as well as for the percentage of NaCl. To evaluate the correlation between ST and ODA among the cultures, the Spearman correlation test was used. All statistical analyses were performed using RStudio software (version 2024.04.2+764) and at 5% significance level.

Results and Discussion

Biosurfactant production and yield

Table 1 shows the surface tension values determined after 48 h of cultivation and a reduction in surface tension to 26.6 mN/m can be observed in the co-cultivation. However, the difference with the monoculture of *S. marcescens* was not significant (p=0.539), probably due to the lack of symbiotic interaction between the microorganisms evaluated. The selection of potential inducing partners for the symbiotic interaction of consortia of microorganisms affects the production of biosurfactants (Alves et al., 2019). Nevertheless, studies have previously proven the biosurfactant production capacity of the bacterium *S. marcescens* (Araújo et al., 2019; Santos et al., 2021). A study reported a surface tension of 26.5 mN/m for the monoculture of *S. marcescens*, similar to that obtained in this study, but in 96 h of cultivation—longer than the 48 h of this research (Rosas-Galván et al., 2018). Thus, although co-cultivation did not increase biosurfactant production, it accelerated the reduction of surface tension.

Table 1 – Surface tension and oil displacement area obtained from monocultures and co-cultures of *Serratia marcescens* and *Tetradesmus obliquus*.

Microorganisms	ST (mN/m)	ST reduction (%)	ODA (cm^2)
Serratia marcescens	27.4±0.1 ab*	62	34.54±0.02 a
Tetradesmus obliquus	40.3±0.1 b	44	6.90±0.20 b
Serratia marcescens+Tetradesmus obliquus	26.6±0.1 a	63	50.24±0.02 c

ST: surface tension; ODA: oil displacement area; ±: standard deviation; *Distinct letters represent significant differences between crops according to Dunn's (p<0.05) and Tukey's (p<0.05) tests, respectively.

On the other hand, as demonstrated in Table 1, the co-cultivation showed a significant increase in the ODA of the monocultures of *S. marcescens* and *T. obliquus* ($p<0.001$). In the Spearman correlation test, a negative correlation ($p=0.9108$; $p=0.0006$) was observed between ST and ODA, evidencing that the lower the surface tension achieved by a culture, the higher the ODA values (Table 1). Table 2 summarizes the results of biosurfactant production by co-cultivation of *S. marcescens* and *T. obliquus* performed in this study, compared to others previously obtained by monoculture of *S. marcescens* in submerged fermentation. It is noteworthy that this comparison was performed only with monoculture of *S. marcescens*, as there is no report in the literature of biosurfactant production by monoculture of the microalgae *T. obliquus* or by co-cultivation of *T. obliquus* with *S. marcescens*. Previous studies have shown higher surface tension values in incubation periods equal to or greater than those observed in the current research, under mechanical agitation conditions, which results in a higher production cost (Roldán-Carrillo et al., 2011; Montero-Rodríguez et al., 2015; Almansoory et al., 2017; Huang et al., 2020; Chen et al., 2024). On the other hand, the emulsification index with burnt engine oil was 96%, standing out among the studies presented in Table 2.

Figure 1 illustrates the dispersant activity of the biosurfactant produced by *S. marcescens* and *T. obliquus* in monoculture and co-culture. The ODA of the biosurfactant produced by *S. marcescens* in monoculture (Figure 1D) was 25.12 cm^2, while in co-culture with *T. obliquus*, it was 50.24 cm^2 (Figure 1C)—a value equal to the ODA of the positive control 50.24 cm^2 (Figure 1B). Finally, *T. obliquus* alone (Figure 1E) presented an ODA of only 4.52 cm^2. Therefore, the dispersant potential of the biosurfactant produced by *S. marcescens* in co-cultivation stands out, being identical to the commercial detergent.

This result was also better than those obtained by biosurfactants produced in monoculture by other strains of *S. marcescens* (Elemba et al., 2015; Montero-Rodríguez et al., 2015). Therefore, the present study stands out for proposing a biological dispersant agent with great potential for application in oil spill bioremediation processes. Luo et al. (2021) also reported the bioremediation capacity using a mixed consortium of bacteria and microalgae.

Table 2 – Comparison of biosurfactant production by co-culture of *Serratia marcescens* and *Tetradesmus obliquus* carried out in this study, with the literature using monocultures of *Serratia marcescens* in submerged fermentation.

Microorganisms	Substrates	Growing conditions	Surface tension (mN/m)	Interfacial tension (mN/m)	Emulsification index (%)	Yield of crude biosurfactant (g/L)	Reference
Serratia marcescens UCP 1591 and *Tetradesmus obliquus* A5F5402	wheat bran+waste frying soybean oil	time: 48 h temperature: 28°C agitation: no aeration: yes	26.6	1.0	96.0 (burnt engine oil)	1.75	Present study
Serratia marcescens SMΔR	glucose+peptone	time: 24 h temperature, agitation, and aeration: no	32.0	6.0	<80.0 (kerosene and diesel)	1.17	Chen et al. (2024)
Serratia marcescens N2	waste frying oil+peptone	time: 48 h temperature: 28°C agitation: 150 rpm aeration: no	25.7	-	90.0 (residual frying oil)	9.40	Elkenawy and Gomaa (2022)
Serratia marcescens ZCF25	olive oil	time: 72 h temperature, agitation, and aeration: no	29.5	-	56.6 (n-hexane) 55.6 (mineral oil)	-	Huang et al. (2020)
Serratia marcescens SM3	glycerol+peptone+ammonium sulfate	time: 96 h temperature: 30°C, agitation: 150 rpm aeration: no	26.5	-	79.9 (kerosene)	4.80	Rosas-Galván et al. (2018)
Serratia marcescens	glycerol+peptone+ammonium sulfate	time: 120h temperature: 30°C, agitation: 200 rpm aeration: no	28.4	-	-	1.42	Almansoory et al. (2017)
Serratia marcescens UCP 1549	cassava wastewater+ post-frying corn oil	time: 72 h temperature: 28°C agitation 150rpm aeration: no	27.8	-	72.7 (burnt engine oil) >60 (engine oil and diesel)	-	Montero-Rodríguez et al. (2015)
Serratia marcescens	Glucose	time: 48 h temperature: 30°C agitation: 100 rpm aeration: 0.1 vvm	31.3	1.81	71.4 (kerosene)	21.60	Roldán-Carrillo et al. (2011)

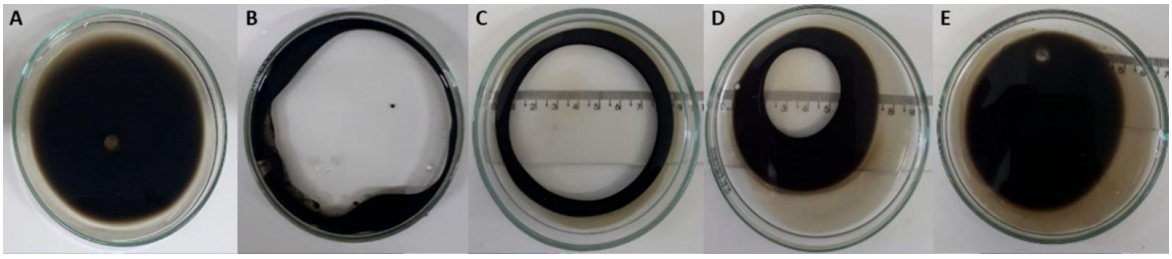


Figure 1 – Dispersion area of burnt engine oil from biosurfactant produced in monoculture and co-culture of *Serratia marcescens* and *Tetradesmus obliquus*: (A) distilled water (negative control); (B) commercial detergent (positive control); (C) crude biosurfactant produced by co-cultivation; (D) crude biosurfactant produced by *Serratia marcescens*; and (E) crude biosurfactant produced by *Tetradesmus obliquus*.

Considering the best ODA values obtained in co-cultivation, this condition was investigated to determine the interfacial tension and emulsification index. The interfacial tension of the crude biosurfactant produced by co-cultivation was 1 mN/m and the EI_{24} was 96%—excellent results when compared with those previously reported for biosurfactants produced by monoculture of *S. marcescens* (Almansoory et al., 2017; Chen et al., 2024) and for the chemical surfactant SDS (7 mN/m) (Pereira et al., 2013). These interfacial tension and emulsification values are in correspondence with the ODA value verified above, since the basic principle of the action of a dispersant in contact with oil or any hydrophobic compound is to reduce the droplet size, decreasing the interfacial tension between oil and water. Thus, an emulsification process occurs, where an effective dispersant converts the oil slick into discrete droplets that remain stable until coalescence (Araújo et al., 2019).

According to the results obtained in this research, the effectiveness of co-cultivation for biosurfactant production was evident, in agreement with another study which found an increase in biosurfactant activity when *S. marcescens* V1 was co-cultivated with the bacterium *Bacillus pumilus* (Dusane et al., 2011). However, the authors performed only the drop collapse test to evaluate the potential for biosurfactant production.

To verify the biosurfactant yield in the present study, the acid precipitation method was used, which recovered a brown compound after centrifugation. This method was chosen because it was more effective at isolating the biosurfactant without the presence of pigment (Santos et al., 2021). Previously, they also used acidification to pH 2 to isolate the biosurfactant produced by *Serratia* strains, confirming the suitability of this recovery technology (Almansoory et al., 2019).

The yield of the biosurfactant produced by the co-cultivation of *S. marcescens* and *T. obliquus* was 1.75 g/L after 48 h of fermentation. This result was slightly higher than that reported by Almansoory et al. (2017), 1.42 g/L in 120 h, which means, in a period longer than that of the present study. Studies on the production of biosurfactants by *S. marcescens* strains reported cultivation times of 72 h (Montero-Rodríguez et al., 2015, Huang et al., 2020), 96 h (Rosas-Galván et al., 2018), and 120 h (Santos et al., 2021; Chen et al., 2024), which are longer than in the present study (Table 2). A significant result in a shorter time reduces possible contamination and high production costs. Among the advantages of co-cultivation about the other fermentation methods analyzed, the following are noteworthy: shorter cultivation time and energy consumption when carried out in synergy of microorganisms, which occurs naturally with the exchange of gases between them (Crespo et al., 2023).

Preliminary characterization of the biosurfactant

The analysis of the Zeta potential of the biosurfactant obtained by co-cultivation showed an anionic character (-67.0 mV), corroborating other biosurfactants produced by *S. marcescens* strains (Araújo et al., 2019; Santos et al., 2021). Anionic surfactants have a negative charge on their hydrophilic heads, besides strong detergent, foaming, and wetting power. Therefore, they are widely used in the formulation of cosmetics, cleaning products, and the removal of hydrocarbons from soils and sediments (Pruthi and Cameotra, 2020; Santos et al., 2021).

On the other hand, the biochemical composition determination of the biosurfactant indicated a lipopeptide nature (lipids 53%, proteins 30%, and carbohydrates 17%), which was confirmed by the FTIR analysis of the isolated biomolecule. According to the infrared spectrum shown in Figure 2, a broad stretching peak was observed around 3265.49 cm^{-1}, corresponding to the N–H stretching, which indicates the peptide groups in the molecule characteristic of biosurfactant with lipopeptide nature. The adsorption peaks detected at 2924.09 cm^{-1} and 2852.72 cm^{-1} were attributed to the presence of methyl and methylene groups, respectively, due to the stretching vibration of the C–H groups (Rosas-Galván et al., 2018; Santos et al., 2021). Another significant peak at 1654.92 cm^{-1} was associated with the N–CO bond stretching characteristic of the amide group (Rajitha et al., 2024). The band with the highest absorbance, found at 1101.35 cm^{-1}, can be correlated with the C–O–C vibrations in esters (Zehra et al., 2023).

The identified peaks corroborated the lipopeptide nature of the biosurfactant produced by the co-cultivation of *S. marcescens* with *T. obliquus*, in agreement with biosurfactants previously produced by the monoculture of *S. marcescens* (Roldán-Carrillo et al., 2011; Huang et al., 2020; Santos et al., 2021; Elkenawy and Gomaa, 2022).

Biosurfactant stability

Figure 3 shows the effects of temperature, pH, and NaCl concentration on the stability of the biosurfactant produced by co-cultivation of *S. marcescens* and *T. obliquus*.

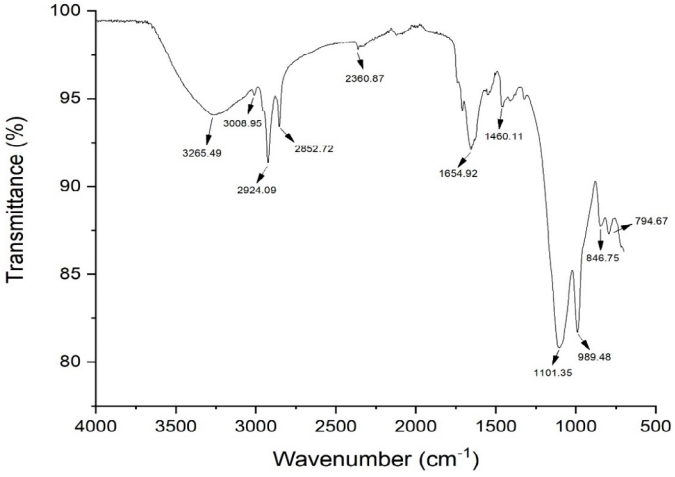
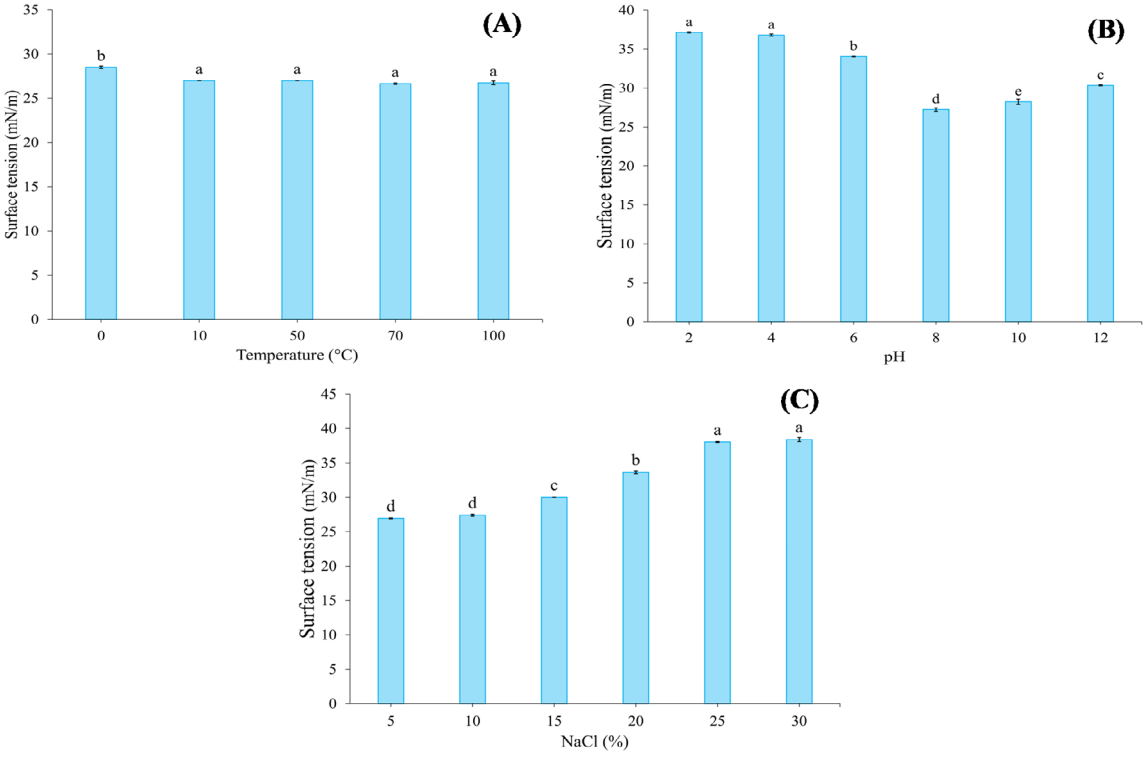


Figure 2 – Fourier transforms of the infrared spectrum of biosurfactant produced by the co-cultivation of *Serratia marcescens* and *Tetradesmus obliquus*.

The results show that the ST did not present a significant difference (p=0.2602) between 10 and 100°C (Figure 3A), with a variation between 27.0 and 26.9 mN/m, demonstrating the thermostability of the biosurfactant in this temperature range. This property is very attractive for industrial processes and environmental applications at extreme temperatures (Alara et al., 2024; Souza et al., 2024).

However, a different behavior was observed against variations in pH and salinity. In Figure 3B, it can be noted that the values for ST (27.25–30.35 mN/m) at alkaline pH (8–12) were significantly lower than those found at acidic pH (2–6) (p=0.0051), indicating greater stability at alkaline pH. The increase in ST at acidic pH may have been caused by the precipitation of the biosurfactant (Santos et al., 2016). Marajan et al. (2018) also identified a positive effect on biosurfactant stability at higher pH (6, 8, 10, and 12).

In turn, regarding the effect of salinity (Figure 3C), significantly lower ST values (26.95 to 30.0 mN/m) were observed at NaCl concentrations between 5 and 15%, compared to concentrations between 20 and 30% (p=0.0051), indicating stability at salinity levels of up to 15%. Although from 20% NaCl, there was an increase in ST to 33.65 mN/m, the results were promising, since biosurfactants commonly tolerate saline concentrations of up to 10% (*w/v*), while synthetic ones are inactivated at concentrations ≥2% NaCl (Pele et al., 2019; Channashettar et al., 2022). Furthermore, the salinity of seawater is around 3%, allowing the application of this biosurfactant in marine environments (Mgbechidinma and Zhang, 2024).



*Different letters represent statistically significant differences.

Figure 3 – Stability of the biosurfactant produced by co-cultivation of *Serratia marcescens* and *Tetradesmus obliquus* under the influence of temperature (A), pH (B), and sodium chloride concentrations (C). Error bars represent the standard deviation.

Phytotoxicity of biosurfactant

Table 3 shows the phytotoxic effect of the biosurfactant produced by the co-cultivation on lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*) seeds. According to the results, the tested concentrations of biosurfactant did not present an inhibitory effect on root elongation or seed germination since the germination index value ≥80% indicates the absence of phytotoxicity (Tiquia et al., 1996). Only the 1% concentration showed a slight decrease in the germination rate of lettuce seeds (79%); although secondary root growth and leaf emergence occurred in all tested solutions, which is in agreement with the literature (Nalini and Parthasarathi, 2014; Araújo et al., 2019).

Ecological compatibility is one of the advantages of biosurfactants when compared to those of synthetic origin (Pereira et al., 2013; Akbari et al., 2018), constituting an essential requirement for environmental application. In this sense, toxicity assessment is considered an important factor in predicting the potential effects of biosurfactants on ecosystems (Nalini and Parthasarathi, 2014; Selva Filho et al., 2023). Phytotoxicity tests using vegetable seeds are commonly performed to investigate the toxic potential of microbial surfactants (Araújo et al., 2019; Selva Filho et al., 2023).

Table 3 – Phytotoxicity of the biosurfactant produced by co-cultivation of *Serratia marcescens* and *Tetradesmus obliquus* on lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*) seeds. Germination indices are presented as mean ± standard deviation of three independent experiments.

Vegetable seeds	Germination index (%)		
	Biosurfactant concentration (%)		
	0.1	0.5	1
Lactuca sativa	97.7±0.15	80.0±1.0	79±0.6
Cucumis sativus	86.7±0.20	83.3±0.3	83±0.2

Application of biosurfactant to remove petroleum derivatives from mollusk shells

Figure 4 demonstrates the ability of the biosurfactant produced by the co-cultivation of *S. marcescens* and *T. obliquus* to remove burnt engine oil from mollusk shells. It can be seen that the biosurfactant could remove 100% of the impregnated oil, indicating its effectiveness in decontaminating solid surfaces impacted by oil spills in the marine ecosystem. Santos et al. (2021) verified the removal of 100% of burnt engine oil from marine rocks using the biosurfactant produced by *S. marcescens* UCP 1549. Contamination by oil derivatives is a major concern due to the damage to marine ecosystems, causing contamination of water bodies and impacting marine life, including fish and mollusks (Sharma et al., 2024).

Conclusions

This study is the first report on the co-cultivation of *S. marcescens* and *T. obliquus* using agro-industrial substrates to produce biodispersants. The results highlighted the suitability of this innovative strategy for the production of a sustainable, high-value compound, with stability under extreme conditions of temperature, pH, and salinity, non-toxic and efficient in the removal of burnt engine oil from mollusk shells. Therefore, the co-cultivation between *S. marcescens* and *T. obliquus* is a promising strategy for the development of a biodispersant with potential application in the bioremediation of marine environments contaminated by petroleum derivatives, which cause economic loss and significant damage to marine ecosystems and coastal communities.

Figure 4 – Removal of burnt engine oil impregnated in mollusk shells by biosurfactant produced by co-cultivation of *Serratia marcescens* and *Tetradesmus obliquus*: (A) Mollusk shell before contamination (control); (B) mollusk shell contaminated with burnt engine oil; and (C) mollusk shell after washing with biosurfactant.

Authors' contributions

Santos, R. A.: conceptualization, formal analysis, investigation, methodology, writing — original draft, writing — review & editing. **Rodríguez**, D. M.: investigation, methodology, writing — original draft. **Mendonça**, R. S.: formal analysis, investigation, methodology. **Takaki**, G. M. C.: conceptualization, resources, validation, visualization. **Porto**, A. L. F.: conceptualization, resources, validation, visualization. **Lima**, M. A. B.: funding, supervision, visualization, writing — original draft, writing — review & editing. **Bezerra**, R. P.: conceptualization, funding, resources, supervision, validation, visualization, writing — original draft, writing — review & editing.

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