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BIOSURFACTANT PRODUCTION BY *Pseudomonas aeruginosa* MSIC02 IN CASHEW APPLE JUICE USING A 2⁴ FULL FACTORIAL EXPERIMENTAL DESIGN

Abstract

In this work, the production of biosurfactants from cashew apple juice by Pseudomonas aeruginosa MSIC02 was investigated by carrying out a 2⁴ full factorial experimental design, using temperature, glucose concentration from cashew apple juice, phosphorous concentration and cultivation time as variables. The response variable was the percentage of reduction in surface tension in the cell-free culture medium, since it indicates the surface-active agent production. Maximum biosurfactant production, equivalent to a 58% reduction in surface tension, was obtained at 37 °C, with glucose concentration of 5.0 g/L and no phosphorous supplementation. Surface tension reduction was significant, since low values were observed in the cell-free medium (27.50 dyn/cm), indicating that biosurfactant was produced. The biosurfactant emulsified different hydrophobic sources and showed stability in the face of salinity, exposure to high temperatures and extreme pH conditions. These physiochemical properties demonstrate the potential for using biosurfactants produced by P. aeruginosa MSIC02 in various applications.

Keywords: biosurfactant, surface tension, Pseudomonas aeruginosa, full factorial experimental design, cashew apple juice, enhanced oil recovery.

Surfactants constitute a very important class of chemical compounds widely used in a variety of industrial sectors, because they act as dispersants and/or solubilizing agents of organic compounds. Most of the commercially used surfactants are synthesized from petroleum derivatives [1]. Biosurfactants are complex biological molecules, which display properties similar to those of the well-known synthetic surfactants, and have been reported as being produced both on the microbial cell surface and excreted extracellularly [2]. They include microbial compounds that exhibit surfactant properties, *e.g.*, polysaccharide-protein complexes, lipopeptides, fatty acids, glycolipids, phospholipids and neutral lipids [3,4].

The most important advantage of biosurfactants over chemical surfactants is probably their ecological

sustainability. Biosurfactants are biodegradable and thus, problems of toxicity and accumulation in natural ecosystems are avoided. They have received more attention lately due to their low toxicity, biodegradability and effectiveness in improving the solubility and biodegradation of hydrophobic compounds [2]. Some potential applications include bioremediation of water-insoluble pollutants, enhanced oil recovery and use in health care and crude oil drilling industries [5,6]. Other potential applications can be found in agriculture, cosmetics, pharmaceuticals, detergents, food processing industries, among others [7,8]. Glycolipids, consisting of hydrophilic carbohydrate and long chain aliphatic acids or hydroxyl-aliphatic acids, are the most common class of the microbially-produced surface active compounds [9]. Of this class, rhamnolipids possess surfactant, antibacterial and antiviral properties [10], as well as spreading abilities, particularly in alkanes.

Rhamnolipids, a glycolipid surfactant containing one or two molecules of rhamnose and 3-hydroxy fatty acids, are produced by bacteria of the genus *Pseudomonas*. Their properties depend on the bacte-

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rial strain, culture conditions and composition of the culture medium [11]. Furthermore, rhamnolipids can be produced using hydrophilic and hydrophobic substrates [12].

Relatively high production costs prevent biosurfactants from being widely employed in the industry. The use of alternative substrates, such as agro-industrial wastes, may be a viable strategy for reducing costs [13]. However, the formulation of culture medium, i.e. determining which waste has the proper nutrients for cell growth and accumulation of the desired product, is a challenge [14]. According to the literature [15], biosurfactant production is controlled by different operating parameters, which should be maintained within a certain range of operational conditions in order to achieve maximum production of biosurfactant. To achieve this goal, the authors suggest the use of a 2^4 full factorial experimental design.

On the north coast of Brazil, cashew apple juice (CAJ) occurs as a residue of the cashew agro-industry, since less than 20% of the total cashew apple (90% of the fruit) is processed by the local industry (beverages and deserts) [16-18]. Furthermore, most of the cashew apple production is left to rot in the soil. These facts, together with its rich composition, make cashew apple juice an interesting and inexpensive (R\$ 1.00/kg) culture medium. However, CAJ characterization indicated that supplementing the juice with essential nutrients is required [19]. Therefore, in this work, nutritional and cultural factors affecting biosurfactant production, in flask-scale, by a new strain of *Pseudomonas aeruginosa* were investigated by using a 2^4 full factorial experimental design. Furthermore, the stability of the biosurfactant relative to some environmental stress conditions (pH, temperature and salinity) was also evaluated.

EXPERIMENTAL

Microorganism

The *Pseudomonas aeruginosa* MSIC02 strain used in this study was isolated from an oil spill off the coast of Ceará. Its rRNA 16S sequence is deposited in the Genbank with the following access number: FJ876297. The culture was maintained on nutrient agar (Biolife) slants at 4 °C and sub-cultured at regular time.

Preparation and characterization of substrate

The physicochemical composition of cashew apple varies widely, depending on the variety, maturation, size, duration of the post-harvesting period, and regional environmental variations [20]. Thus, in

this work, all of the cashew apple juice used was standardized and characterized. In order to obtain consistent results, the same batch of juice was used in all experiments.

Cashew apple juice was extracted by compressing the cashew apple (*Anacardium occidentale* L.). Since fruits of various origins and maturations were used, after compressing, the juice was mixed and centrifuged at 3500 rpm for 20 min (BIO ENG, BE-6000), filtered and stored at -18 °C. The pH of the cashew apple juice was determined using a Tecnal potentiometer (model Tec-3MP) at approximately 27 °C.

Inoculum preparation and biosurfactant production

The bacterial strains were streaked on a nutrient agar (5.0 g/L of peptone, 3.0 g/L of yeast extract and 15 g/L of agar) slant and incubated for 24 h at 30 °C. Three loops of culture were inoculated in 50 mL of nutrient broth (5.0 g/L of peptone and 3.0 g/L of yeast extract) in a 250 mL Erlenmeyer flask and incubated in a rotary shaker (Tecnal - TE240, BR) at 30 °C and 150 rpm for 18-24 h. Afterwards, the optical density (600 nm) of bacterial suspension was adjusted to 0.5 and an aliquot of 1 mL of inoculum (2%, v/v) was transferred to a 250 mL Erlenmeyer flask, containing 50 mL of medium (CAJ), and incubated at 30 °C, 150 rpm in a rotary shaker (Tecnal - TE240, BR). The initial pH of CAJ medium was adjusted to 7.0 with NaOH 1 mol/L. Samples were collected at time-defined intervals and submitted to analysis. During the assays, NaNO₃ was used as an inorganic nitrogen source, in a concentration of 5 g/L. Nutrient broth was sterilized at 121 °C for 15 min. The media containing cashew apple juice (CAJ) were sterilized by filtration using a sterile membrane (Millipore cellulose ester) with a pore diameter of 0.45 µm.

Effect of variables on surface tension

The production of biosurfactants by *P. aeruginosa* MSIC02 in cashew apple juice was carried out by 2^4 full factorial experimental design, using temperature (X_1), glucose concentration from cashew apple juice (X_2), phosphorous concentration through KH₂PO₄ (X_3) supplementation and cultivation time (X_4) as variables. Cashew apple juice was diluted with water in order to achieve the desired glucose concentration. The percentage of reduction in surface tension was adopted as the response variable, since it indicates the surface-active agent production. According to the literature [21-23], the measurement of surface tension may be used to detect biosurfactant production and most of the other methods that measure the surface properties of biosurfactant use surface tension reduction as the standard. A 2^4 full

factorial experimental design was used, with three central points to determine error, made up of 19 experiments (Table 1). The factors studied were select based on the literature [4,24-28]. The minimum and maximum ranges of the variables investigated and the full experimental plan with respect to their values are listed in Table 1. The data were statistically analyzed using Statistica 6.0 (Statsoft Inc., USA) *via* multiple regression analysis using the least squares method, taking into account the isolated terms and interaction of the studied variables.

The results of the experiments were analyzed in order to determine the Pareto chart, the significant variables, the effect, and the intensity of the stationary point, *i.e.*, with the aim of evaluating the existence of a maximum or minimum point.

Determination of the effect of environmental factors on biosurfactant activity

Stability studies were conducted using cell-free broth obtained after 48 h of cultivation [25,29,30]. The pH stability was performed by adjusting the broth to different pH values (2.0-11.0), using 1.0 mol/L NaOH or HCl depending on the desired value. To study the effect of salinity on the stability of biosurfactant, different concentrations of NaCl (0.0-20%, w/v) were added to broth samples and mixed until completely dissolved. Broth samples were heated in a boiling water bath at constant temperature (4, 15, 30, 50 and

100 °C) and in autoclave (121 °C) for 15 min and cooled at room temperature. Samples were also heated in a boiling water bath (100 °C) for different time intervals (0, 15, 30, 60 and 75 min) and cooled at room temperature. After each treatment, the surface tension values were determined.

Analytical methods

Biomass content. Cell growth was determined by measuring the optical density of samples, using a UV-visible spectrophotometer (20 Genesis, BR) at 600 nm. Cell concentration was determined by dry weight by filtering through a 0.45 µm previously weighted Millipore membrane [31].

Carbohydrate concentration. Substrate concentration (glucose and fructose), present in CAJ, was measured by HPLC using a Waters high-performance-liquid chromatograph equipped with a refractive index detector and a Shodex Sugar SC1011 column (8.0 mm×300 mm). Milli-Q water was used as a solvent with a flow rate of 0.6 mL/min at 80 °C. The samples were identified by comparing the retention times with those of carbohydrate standards [19].

Surface tension determination. Surface tension was determined with a Tensiometer (Krüss) at 30 °C, according to the De Nöuy ring method. The determination of surface tension was replicated and it was performed using cell-free supernatants obtained after centrifugation.

Table 1. Results of experimental using four independent variables and three center points showing observed values for biosurfactant production by *P. aeruginosa* MSIC02: X_1 - temperature, X_2 - glucose concentration, X_3 - phosphorous concentration, X_4 - cultivation time, R_1 - surface tension and R_2 - surface tension reduction

Assay	X_1 / °C	X_2 / g L ⁻¹	X_3 / g L ⁻¹	X_4 / h	R_1 / dyn cm ⁻¹	R_2 / %
1	30	5	0	24	31.00±0.0	40.10
2	37	5	0	24	28.00±0.0	47.00
3	30	10	0	24	34.00±0.71	24.44
4	37	10	0	24	28.50±0.0	56.55
5	30	5	1	24	29.50±0.0	51.73
6	37	5	1	24	28.50±0.0	51.07
7	30	10	1	24	38.25±0.49	15.47
8	37	10	1	24	31.10±0.41	30.58
9	30	5	0	72	27.50±0.0	46.86
10	37	5	0	72	27.50±0.0	47.95
11	30	10	0	72	31.00±0.0	41.87
12	37	10	0	72	27.50±0.0	58.08
13	30	5	1	72	30.00±0.0	50.92
14	37	5	1	72	31.00±0.0	46.78
15	30	10	1	72	37.00±0.0	18.23
16	37	10	1	72	28.50±0.0	36.38
17 (C)	33.5	7.5	0.5	48	28.63±0.25	17.21
18 (C)	33.5	7.5	0.5	48	29.00±0.0	18.40
19 (C)	33.5	7.5	0.5	48	29.00±0.0	16.98

Emulsifying activity. Emulsifying activity against gasoline, diesel, hexane, kerosene and soy oil was determined according to Rocha *et al.* [19] and reported as (E_{24}).

Statistical analysis

All the experiments were carried out with three independent replicates. In order to verify significant differences, results were evaluated statistically, at 95% confidence level ($p < 0.05$), using the Analysis of variance (ANOVA) and Tukey multiple comparison tests, available in Microcal Origin 8.1 software (Microcal Software Inc., Northampton, MA, USA).

RESULTS AND DISCUSSION

Biosurfactant production by a 2^4 full factorial experimental design

In a previous work [32], the ability of biosurfactant production by *Pseudomonas aeruginosa* ATCC 10145 in batch cultivation using cashew apple juice (CAJ) and mineral media was evaluated. This initial study indicated that traditional carbon sources for biosurfactant production could be replaced by CAJ, supplemented with peptone. Since the production of biosurfactant on CAJ was demonstrated, further studies were conducted in order to enhance biosurfactant production. For that purpose, and due to the complex nature of biological processes [15], a 2^4 full factorial experimental design was utilized to investigate the reduction in surface tension (biosurfactant production) and to determine the significance of process parameters and their interactions. Table 1 shows the results obtained in the 2^4 full factorial design from the studied variables: temperature (X_1), glucose concentration (X_2), phosphorous concentration (X_3) and cultivation time (X_4), using the isolated

P. aeruginosa MSIC02 strain. Table 1 also shows the surface tension values for the media after fermentation for each experiment.

The results shown in Table 1 indicate that maximum biosurfactant synthesis was obtained in the ninth, tenth and twelfth experiments. A significant reduction in surface tension was observed, once tension of the cell-free medium reached 27.50 dyn/cm. Based on the results, one can conclude that CAJ is also a suitable substrate for the production of biosurfactants by *P. aeruginosa* MSIC02, since the surface tension of the cell-free broth was reduced to below 30 dyn/cm [11,33].

Table 2 shows a regression analysis of the estimates and hypothesis tests for the coefficients of regression. The determination of the significant parameters was performed through a hypothesis test (Student's *t*-test) with a 5% level of significance.

The results of this analysis were also shown using the Pareto chart (Figure 1) and they very clearly present the most significant effects. According to Garrido-Lopez and Tena [34], the length of the bars is proportional to the absolute value of the estimated effects. In this work, the dashed line represents 95% of confidence interval. Effects that cross this line are significant values with respect to the response. In the Pareto chart for surface tension reduction, the significant effect is due to glucose concentration, followed by temperature. Enhancing glucose concentration negatively affects biosurfactant production, while enhancing temperature positively affects biosurfactant production. The negative influence of glucose concentration on biosurfactant production may be explained by the effect caused by inhibitors, such as tannin, which are present in cashew apple juice. The interactions between glucose concentration and phosphorous concentration (X_2X_3), and glucose concen-

Table 2. Results of regression analysis using 2^4 full factorial design

Parameter	Effect	Pure error	$t(2)$	p	Coefficient	Standard error	Level of confidence (95%)	
							Inferior limit	Superior limit
Mean/interc.	37.7158	0.1748	215.6996	0.00002	37.71579	0.1748	215.6996	0.00002
(X_1) Temperature	10.5963	0.3811	27.8056	0.00129	5.29813	0.1905	27.8056	0.00129
(X_2) Glucose	-12.6013	0.3811	-33.0669	0.00091	-6.30063	0.1905	-33.0669	0.00091
(X_3) Phosphorus	-7.7113	0.3811	-20.2350	0.00243	-3.85563	0.1905	-20.2350	0.00243
(X_4) Time	3.7663	0.3811	9.8830	0.01008	1.88312	0.1905	9.8830	0.01008
X_1X_2	9.7988	0.3811	25.7128	0.00151	4.89938	0.1905	25.7128	0.00151
X_1X_3	-3.4813	0.3811	-9.1351	0.01177	-1.74063	0.1905	-9.1351	0.01177
X_1X_4	-2.7688	0.3811	-7.2655	0.01842	-1.38438	0.1905	-7.2655	0.01842
X_2X_3	-12.3588	0.3811	-32.4305	0.00095	-6.17938	0.1905	-32.4305	0.00095
X_2X_4	3.1138	0.3811	8.1708	0.01465	1.55687	0.1905	8.1708	0.01465
X_3X_4	-2.9013	0.3811	-7.6132	0.01682	-1.45063	0.1905	-7.6132	0.01682

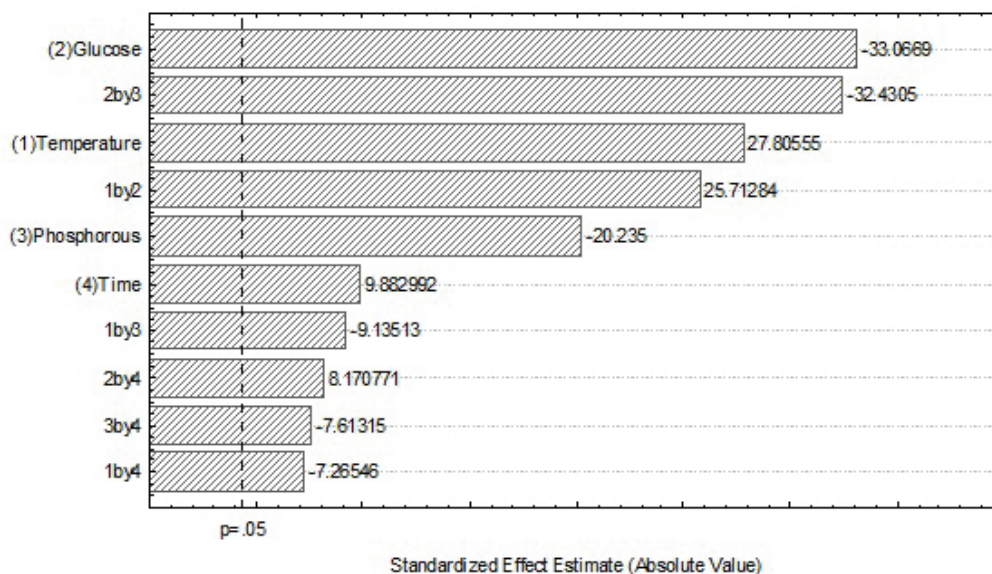


Figure 1. Biosurfactant production by *P. aeruginosa* MSIC02 using CAJ: Pareto chart of standardized effects.

tration and temperature (X_1, X_2), negatively affected biosurfactant production.

Maximum production of biosurfactants was observed, with a 58% reduction in surface tension, at the minimum level (-1) of glucose concentration (5.0 g/L) and the maximum level (+1) of temperature (37 °C) when phosphorous concentration and cultivation time are fixed at their respective center point values of 0.5 g/L and 48 h.

Reduction in surface tension increases with temperature in the range studied, while increasing the glucose concentration lead to a decrease in the values of "reduced surface tension". Therefore, within the experimental data, it can be observed that when there is an increase in glucose concentration from cashew apple juice, a decrease in biosurfactant production occurs. The opposite was observed with temperature, in which a greater decrease in surface tension occurred when the temperature rose from 30 to 37 °C. However, there is not enough significant evidence of the interaction of these two variables with the other two variables in the study (phosphorous concentration and cultivation time). Wei *et al.* [26] also observed that rhamnolipid production by an indigenous *P. aeruginosa* J4 was affected by temperature and agitation rate, being 30 °C and 200 rpm the best conditions for rhamnolipid production. Other authors [35] determined the optimum values of temperature (35 °C) for a rhamnolipid biosurfactant-producing strain (*Pseudomonas aeruginosa* F-2), which is in the range studied in this work for *P. aeruginosa* MSIC02.

According to data in the literature [28], phosphate metabolism can influence biosurfactant pro-

duction, and inorganic phosphate is important for the capacity buffer. Other authors [24,36] mentioned that the carbon/phosphorous (C/P) ratio is important in the production of rhamnolipids by *P. aeruginosa*. Mulligan *et al.* [28] studied the influence of phosphate metabolism on biosurfactant production by *P. aeruginosa* ATCC 9027 using glucose as carbon source and they concluded that a shift in phosphate metabolism coincided with biosurfactant production. However, in the present work, it was observed that the used phosphorous concentration did not significantly influence biosurfactant production.

It was also observed that cultivation time had no expressive influence on biosurfactant production. Low values of surface tension of the cell-free broth were achieved after 24 h of fermentation, independent of the experiment. Therefore, in that time period, the concentration of biosurfactant in the medium was already above critical micelle concentration (CMC) and an increase in the concentration of biosurfactant that was produced would not reduce the surface tension any further. This fact can explain the lack of influence of cultivation time on biosurfactant production. Some authors [37] report the same behavior and explain that even in the presence of a small concentration of biosurfactants, CMC may be achieved, from which no variation in the surface tension can be observed.

Kinetics of biosurfactant production

The kinetics of growth and the production of biosurfactants by *P. aeruginosa* MSIC02 at 37 °C and 150 rpm in an optimized cashew apple juice medium were studied. Several process parameters were

monitored during the fermentation period and the results are presented in Figure 2. It can be observed that the variation in biomass concentration ($\ln(X/X_0)$) is a typical microbial growth curve. A comparison between cell growth, surface tension and substrate uptake allows one to observe that biosurfactant production coincides with the extinguishing of the substrate and the beginning of the stationary phase (Figure 2). According to the literature [38], several biosurfactants were recognized as secondary metabolites, while others were considered growth associated. In this study, the observed behavior is typical of secondary metabolites.

The consumption pattern of glucose and fructose during the fermentation was confirmed by monitoring these sugars (Figure 2). It can be seen that fructose concentration stayed almost constant along fermentation, meaning that only glucose was consumed by the microorganism. The same behavior was observed before when *P. aeruginosa* ATCC 10145 was cultivated in cashew apple juice [32].

The surface tension of the culture broth decreased from 47.7 to 28.0 dyn/cm, and remained constant for up to 72 h (Figure 2). The lowest surface tension was achieved at the stationary phase (48 h). *P. aeruginosa* is known to produce rhamnolipids, which are capable of reducing the surface tension of the media by approximately 30–60% [39]. In this work, when CAJ was used, the surfactant produced by the bacteria was found to reduce the surface tension of

the medium by 58.08% (Table 1), which is comparable with the earlier reports.

Emulsifying activity of the biosurfactant against non-aqueous phase liquids

According to the literature [30], the emulsification properties of any surfactant depend on the tested solvent. Therefore, different non-aqueous phase liquids were tested as a substrate for emulsifying activity by the biosurfactant and the results are shown in Figure 3.

As shown in Figure 3, all of the hydrocarbons and vegetable oil tested served as substrate for emulsification by the biosurfactant, but it showed appreciable emulsification indices (more than 40%) with gasoline, diesel, kerosene and soy oil. Kerosene (83.5 % emulsified) was the best substrate, while hexane (10.7% emulsified) was the poorest. Most microbial surfactants are substrate specific, solubilizing or emulsifying different hydrocarbons at different rates [40,25]. The results of emulsifying activity (high values) indicate potential use, for instance, for enhanced oil recovery. The water-oil emulsions showed to be compact and remained stable for more than 4 months at room temperature, suggesting that the addition of such biosurfactant into a remediation process may enhance the availability of the recalcitrant hydrocarbon. Furthermore, the ability to emulsify vegetable oils suggests a potential application in the pharmaceutical and cosmetic industries.

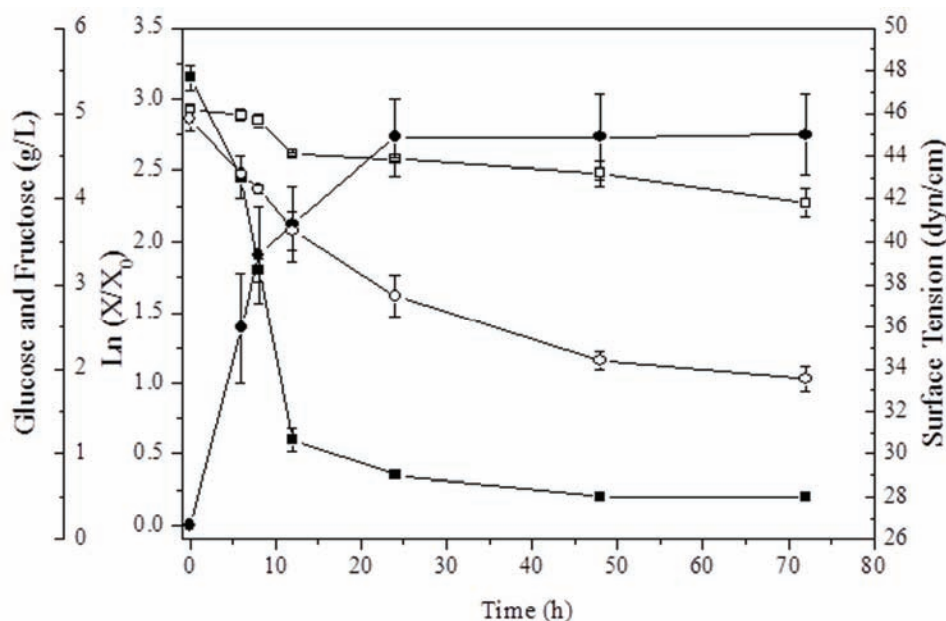


Figure 2. Kinetics of growth and production of biosurfactants by *P. aeruginosa* MSIC02 at 37 °C and 150 rpm in an optimized cashew apple juice medium, contained glucose concentration of 5 g/L and no phosphorous supplementation. (●) Optical density; (■) surface tension; (○) glucose concentration (g/L); (◻) fructose concentration (g/L).

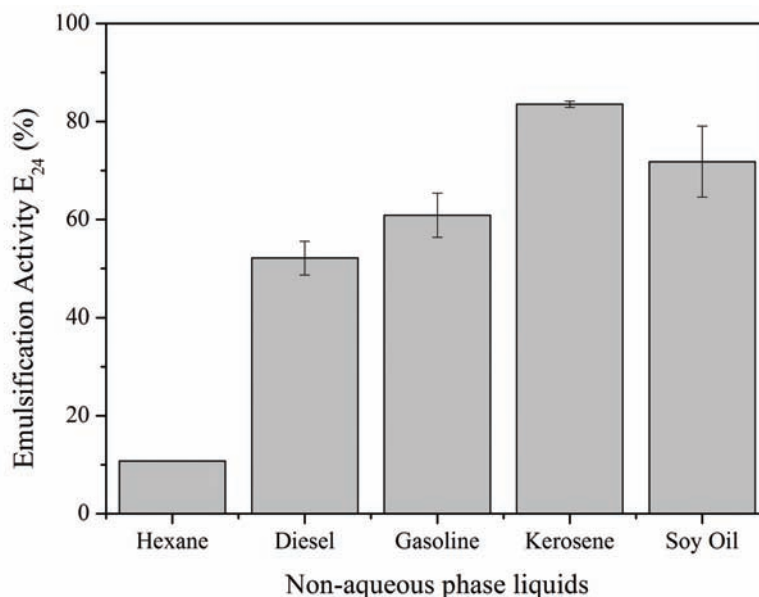


Figure 3. Emulsifying activity (E_{24}) of biosurfactant (produced by *P. aeruginosa* MSIC02 using cashew apple juice) obtained different hydrocarbons and vegetable oil. Error bars represent standard deviations.

Biosurfactant stability analysis

The stability of biosurfactants under extreme conditions is a pre-requisite for their potential applications in enhanced oil recovery, and in environmental and industrial applications [2,3,41,42]. The conditions that affect the performance of a biosurfactant are usually salinity, pH and temperature [30].

The effect of NaCl concentration on the surface activity of the biosurfactant produced is shown in Figure 4A, which was evaluated in order to investigate its applicability in the bioremediation of saline environments. Statistical tests (ANOVA and Tukey multiple comparison) showed that the surface activity was not significantly influenced by NaCl concentrations ranging from 0 to 10% and 20%, w/v, as shown in Figure 4B. The surface tension presented a significant decreased when 15%, w/v, NaCl was added, which may be explained by the presence of a net negative charge in the solution/air interface. Helvacı *et al.* [43] stated that electrolytes directly affect the carboxylate groups of the rhamnolipids. At pH 6.8, carboxylic acid groups are ionized and strong repulsive electrostatic forces between rhamnolipid molecules is promoted. As a result, a decrease in surface tension values is observed, probably because of the formation of a close-packed monolayer, caused by the fact that the negative charge is shielded by Na^+ in the electrical double layer in the presence of NaCl [25,43].

Although surface tension was significant affected by NaCl concentration, it is important to notice that the surface tension remained in range of 29-30

dyn/cm, meaning that the biosurfactant retained its surface activity. Even higher salt tolerance (up to 35%) has been observed for biosurfactants obtained from a marine *P. aeruginosa* [44]. Considering that 3% is the highest salinity of sea in the world [25], these results indicate that the biosurfactant from *P. aeruginosa* MSIC02 could be applied in contaminated marines.

The effect of pH on biosurfactant activity is shown in Figure 4B. The surface activity was retained over a pH range of 6-11 with minimal deviation in surface tension, but the maximum surface activity was reached at pH 7-9, showing no significant difference in the surface tension. The rhamnolipids have their optimum aqueous solubility at neutral to alkaline pHs, which is attributed to their acidic nature, once the reported pK_a is 5.6 [25]. The stability loss at low pH scale (<5) is probably due to the occurrence of precipitation, caused by the consequent insolubility of the biosurfactant produced by *P. aeruginosa* at these pH values [21,41,45]. It has been reported that as the pH increases from 5 to 8, the negative charge of the polar head increases, promoting an increase in aqueous solubility [46].

Studies on the effect of heat treatment demonstrated that no appreciable change in surfactant surface activity had occurred (Figure 4C). Although surface tension means are significantly different, the surface tension values (28-30 dyn/cm) remained stable after exposure to high temperatures (100 °C), even after 75 min. The stability of the biosurfactant

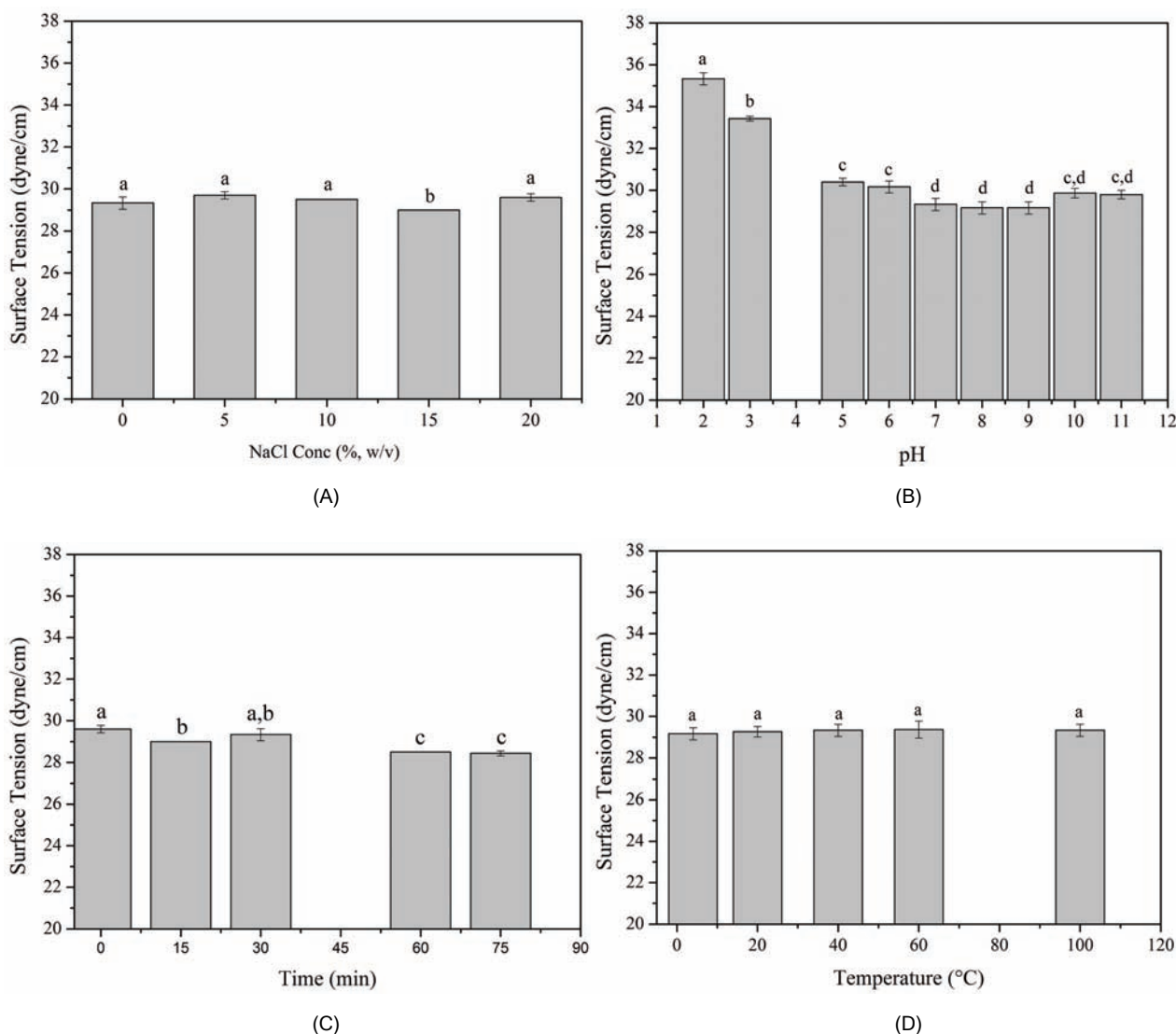


Figure 4. A) Effect of NaCl on activity of biosurfactant, B) effect of pH on activity of biosurfactant, C) effect of time of exposure at 100 °C and D) effect of temperature on activity of biosurfactant produced by *P. aeruginosa* MSIC02 at 37 °C and 150 rpm in an optimized cashew apple juice medium. Values with different letters present statistically significant differences ($p < 0.05$).

was also tested over a wide temperature range. The biosurfactant produced showed to be stable during incubation for 15 min at temperatures ranging from 4 to 100 °C (Figure 4D), with no significant difference on surface tension values. When submitted to autoclave sterilization (121 °C/15 min), the surface activity was also maintained (29.1±0.1 dyn/cm). These results indicate the usefulness of the produced biosurfactant in industries where heating to achieve sterility is of paramount importance.

Likewise, thermo-tolerant and thermo-stable biosurfactants from *P. aeruginosa* [25,46], *Bacillus subtilis* [3,30] and from *Bacillus licheniformis* [47] have been reported. The biosurfactant that was produced in this study showed stability when exposed to high temperatures, indicating that it could be used under

extreme temperature conditions, such as in microbially enhanced oil recovery - MEOR [46]. Emulsifying biosurfactants that are stable in environments with high pH and salinity would find applications in the bioremediation of spills at sea. Furthermore, the biosurfactant may also be useful for bioremediation in hot and slightly alkaline environments.

CONCLUSION

Through the 2⁴ full factorial design, it was observed that of the 4 variables studied, glucose concentration and temperature were the most significant for biosurfactant production by *Pseudomonas aeruginosa* MSIC02. Increasing the cultivation temperature and reducing the glucose concentration, present

in the cashew juice, caused a greater reduction in surface tension, indicating greater biosurfactant production. In addition, the properties (minimum surface tension and emulsifying activity) of the produced biosurfactant, as well as its high stabilities at high salinities, elevated temperatures, and over a wide pH range, makes these biosurfactants potential candidates to be used in bioremediation of contaminated sites and in the petroleum industry (MEOR) where drastic conditions are very common.

Nomenclature

C/P	Carbon/phosphorous ratio
CAJ	Cashew apple juice
CMC	Critical micellar concentration
E_{24}	Emulsifying activity, %
X_1	Temperature
X_2	Glucose concentration from cashew apple juice
X_3	Phosphorous concentration (KH_2PO_4)
X_4	Cultivation time

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NAUČNI RAD

PRODUKCIJA BIOSURFAKTANTA POMOĆU *Pseudomonas aeruginosa* MSIC02 NA PODLOZI SA SOKOM JABUKE KAŽUJE POMOĆU PUNOG FAKTORIJELNOG EKSPERIMENTALNOG PLANA 2⁴

Produkcija biosurfaktanta pomoću Pseudomonas aeruginosa MSIC02 u podlozi sa sokom jabuke kažu je ispitivana pomoću punog faktorijskog eksperimentalnog plana 2⁴ u kome su nezavisne promenljive: temperatura, koncentracija glukoze (iz soka jabuke kažu je), koncentracija fosfora i vreme kultivacije. Zavisna promenljiva je bilo procentno smanjenje površinskog napona fermentacione tečnosti bez suspendovanih ćelija, kao indikatora produkcije površinski aktivnog agensa. Maksimalna produkcija biosurfaktanta, ekvivalentna smanjenju površinskog napona od 58%, dobijena je na 37 °C pri koncentraciji glukoze 5,0 g/L i bez dodatka fosfora. Smanjenje površinskog napona je bilo značajno budući da je mala vrednost izmerena u hranljivoj podlozi (27,50 dyn/cm), što je ukazalo na produkciju biosurfaktanta. Ovaj biosurfaktant emulguje različite hidrofobne supstance i pokazuje stabilnost prema salinitetu, visokim temperaturama i ekstremnim pH vrednostima. Ove fizičko-hemijske osobine pokazuju potencijal za različite primene biosurfaktanta proizvedenog pomoću P. aeruginosa MSIC02.

Ključne reči: biosurfaktant, površinski napon, Pseudomonas aeruginosa, puni faktorijski eksperimentalni plan, sok jabuke kažu je, uvećano izdvajanje ulja.