REMOVAL OF PETROLEUM OIL IN SOIL USING BIOSURFACTANTS PRODUCED BY *STARMERELLA BOMBICOLA*

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ABSTRACT

Biosurfactants are widely studied compounds worldwide due to their biodegradability and environmental compatibility, unlike their petrochemical (synthetic) counterparts. This study investigated the biotechnological potential of the yeast *Starmerella bombicola* ATCC22214 in biosurfactant production and its application in oil removal. An experiment was conducted in a 1 L bioreactor containing mineral medium with olive oil, inoculated with 10% yeast, and incubated at 30°C, pH 6.5, 0.5 vvm, 200 rpm for 8 days. The surface tension, yield, biomass produced, emulsification index, dispersion capacity, phytotoxicity, CMC, and chemical composition were analyzed. Additionally, the biosurfactant was applied in oil removal in soil. The produced biosurfactant had a chemical composition of 53% carbohydrates and 45% lipids, was non-toxic, reducing the surface tension of the medium from 62.40 ± 0.16 mN/m to 31.08 ± 0.08 mN/m, with a yield of 8.5 g/L, and a critical micelle concentration (CMC) value of 0.65 g/L, emulsification activity ranging from 99.00% ± 0.1 to 93.75% ± 0.25. The biosurfactant was also able to remove 89.2% ± 0.02 of the petroleum from the sand in a static test. The results indicate the potential of the biosurfactant for mobilizing hydrocarbons in the soil.

Keywords: *Starmerella bombicola*. Biosurfactants. Petroleum.

1 INTRODUCTION

Accidents caused by leaks during petroleum oil extraction, as well as the rupture of underground storage tanks, damage to pipelines, petroleum oil spills, and the disposal of petroleum oily wastewater from ships, contaminate water and soil. [1]. This results in the release of petroleum hydrocarbons into the environment, affecting a large area. These durable and stable contaminants remain in the environment for long periods and do not easily degrade.[2]. One possible solution for the low availability of hydrophobic pollutants is the use of biosurfactants. These compounds consist of molecular structures derived from metabolites mainly produced by bacteria and yeasts, although some fungi also produce them. They also present numerous advantages over chemically derived surfactants, such as low toxicity, stability across a wide pH range and high temperatures, as well as resistance to high saline concentrations.

Therefore, biosurfactants are promising for environmental remediation, including cleaning up oil spills and treating wastewater, by acting on the hydrophobicity of the microbial cell surface, which is an essential aspect of the biodegradation process. Biosurfactants can also enhance the biodegradation of hydrocarbons by emulsifying the contaminant. The aim of this study was to produce a biosurfactant from the yeast *Starmerella bombicola* ATCC 22214, evaluate its properties, potential toxicity, and safe application in remediating soils contaminated with petroleum oil.

2 MATERIAL & METHODS

Production media and culture conditions: The biosurfactant production was carried out by the yeast in a 1L Bioreactor, using the medium described by Konishi [3]., consisting of 50 g of olive oil as a hydrophobic source, 25.0 g of glucose, 1.0 g of yeast extract, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$, 20 g of agar, and 1000 mL of distilled water. The medium was dissolved and sterilized in an autoclave for 20 minutes at 121°C. Subsequently, the production was carried out under orbital agitation at 250 rpm, aeration rate of 0.5 vvm, at a temperature of 30°C for 8 days. Samples were taken every 24 hours during the fermentation to determine biomass, surface tension, biosurfactant yield, and pH.

Análise do biodetergente: After the fermentation period, the cell-free fermentative broth was analyzed for surface tension using a KSV Sigma 700 tensiometer (Finland) with a NUOY ring. Subsequently, the emulsification activity was determined following the method described by Cooper and Goldenberg [4]., and phytotoxicity tests with vegetable seeds were conducted according to Tiquia [5]. In addition, the biodetergent was isolated using ethyl acetate and isopropanol (8:2, v/v), following the procedure described by Daverey & Pakshirajan [6]., and the Critical Micelle Concentration (CMC) and chemical composition of the isolated biosurfactant were determined, following the procedures described by Joslyn [7]., Bligh-Dyer [8]., Somogy-Nelson [9]., and Dubois [10].,while the dispersal or aggregation capability of oil stains was simulated in the laboratory by contaminating seawater samples with 5% Petroleum, following the procedure described by Saeki [11].

Experiments of removal of Petroleum oil adsorbed to sand by biosurfactant in packed columns through static test: Experiments were conducted to remove oil adsorbed to sand by biosurfactant in packed columns through static testing. Glass columns (55 x 6 cm) were filled with approximately 200 g of sand contaminated with 10% of Petroleum oil. The surface was then flooded with 200 mL of biosurfactant solution at 0.325 g/L (Condition 1) and 0.65 g/L (Condition 2). A column containing sand and 200 mL of water was used as a control. The percolation of biosurfactant solutions was evaluated after 24 hours. [12].

3 RESULTS & DISCUSSION

Nowadays, the quality of a new surfactant agent is typically assessed through measurements of surface tension and emulsifying capacity. Research indicates that the surfactant produced by the yeast *Starmerella bombicola* ATCC 22214 in a 1L bioreactor showed a surface tension of 31.08 mN/m, with yeast cell growth reaching 1.0 g/L biomass and pH 6.2 at the end of fermentation. The biosurfactant extraction process yielded 8.5 g/L, with a critical micelle concentration (CMC) value of 0.65 g/L using solvents (ethyl acetate and isopropanol). Furthermore, the surfactant exhibited the ability to form stable emulsions with high efficiency on motor oil (93.75% ± 0.25) and petroleum (99.00% ± 0.1), followed by Biodiesel (90.50% ± 0.2), showing promise for applications in remediation processes where these types of oils are contaminants. Biosynthesis of biosurfactants from yeasts involves structures containing a variety of hydroxylated fatty acids and carbohydrates, characterized by unique surfactant properties. Preliminary analysis of the biosurfactant produced by *S. bombicola* ATCC 22214 revealed a glycolipid molecule consisting of 53% carbohydrates and 45% lipids.

The toxicity test evaluations of the biosurfactant were conducted using microcrustacean larvae (*Artemia salina*) and seeds of the Heart-of-Beef Cabbage (*Brassica oleracea*) and cherry tomato (*Solanum lycopersicum*) plants to ensure that the biosurfactant will not be toxic to the environment. The biosurfactant, at the tested concentrations (0.325 g/L, 0.65 g/L, and 1.3 g/L), yielded satisfactory results, with germination rates ranging from 55 to 90%, demonstrating that the biosurfactant is non-toxic to the plants used in the tests (non-toxic) (Table 1).

Table 1. Phytotoxicity testing at various concentrations of biosurfactant.

Vegetable seeds used	Germination Rate Index (%)			
	Biosurfactant Concentration			
	0.325 g/L	0.65 g/L	1.3 g/L	Água
Cherry tomato *(Solanum lycopersicum var. cerasiforme)*	85.00 ± 0.2	80.00 ± 0.3	55.00 ± 0.2	100.00 ± 0.1
Heart-of-Beef Cabbage *(Brassica oleracea)*	90.00 ± 0.1	80.00 ± 0.2	60.00 ± 0.2	100.00 ± 0.1

Another parameter widely used to determine the quality of a biosurfactant is the evaluation of its dispersing capacity. [13;15]. Therefore, the performance of biotensides as oil dispersants for different oil volumes was assessed, as illustrated in Figure 1. It was observed that the biosurfactant at its critical micelle concentration (CMC) of 0.65 g/L was able to disperse 3 times the initial oil diameter, for a 1:1 (v/v) ratio of biosurfactant to motor oil, indicating that the biosurfactant is an excellent hydrocarbon dispersant and can also be used as a bioremediator in marine environments.

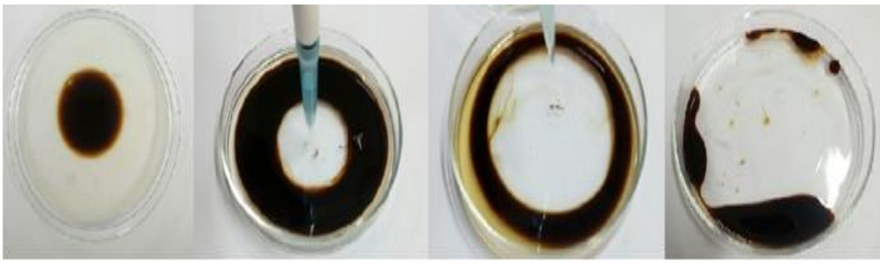

Figure 1 Illustration of petroleum oildroplet dispersed by the action of biosurfactants produced by *S. bombicola* ATCC 22214.

Figure 2 presents the results of experiments on the removal of adsorbed petroleum oil in sand using a biosurfactant from *S. bombicola* ATCC 22214 in packed columns through static tests. After 24 hours of percolation, the isolated biosurfactant achieved a removal rate of 89.2% ± 0.02, while water used as a control only managed to remove 5.2% of the contaminant. This removal rate is promising as the biosurfactant at its CMC was able to eliminate more than half of the oil adsorbed in the sand Silva. [14]. reported a removal of 79.55% of motor oil impregnated in the soil using the crude biosurfactant produced by *Pseudomonas aeruginosa*.

Figure 2 Removal of adsorbed petroleum oil in sand through the bioremediation process using biosurfactant produced by *S. bom*bicola ATCC 22214 in packed columns via static test.

4 CONCLUSION

The yeast *Starmerella bombicola* ATCC 22214 shows great biotechnological potential in biosurfactant production. Furthermore, the results of emulsification experiments, lack of toxicity, and ability to disperse oil in aqueous media clearly demonstrate the feasibility of using the biosurfactant as a biotechnological additive for remediating petroleum oil and derivative-contaminated aqueous and terrestrial environments.

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