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BIOCONVERSION OF CRUDE OIL ALSPHALTENE-FREE FRACTION INTO BIOSURFACTANTS USING *YARROWIA LIPOLYTICA* AS BIOCATALYST

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ABSTRACT

Crude oils are complex mixtures of organic molecules, of which asphaltenes are the heaviest components. Offshore exploration exposes the marine environment to a potential risk of accidents, such as oil spillage. *Yarrowia lipolytica* has emerged as a promising agent in the bioconversion of oils due to its ability to utilize hydrophobic substrates as a carbon source efficiently. The present work evaluated the conversion of an asphaltene-free fraction extracted from crude oil using a natural solvent, into biosurfactants using an *Y. lipolytica* strain isolated from Guanabara Bay. The emulsification index (EI) detected in the medium with asphaltene-free fraction was 35% higher than without it, although greater IE productivity was obtained in the control. The results show the potential to use asphalthene-free fraction from oil spills to produce biosurfactant by *Y. lipolytica*.

Keywords: *Yarrowia lipolytica*. Crude oils. Biosurfactants. Bioconversion.

1 INTRODUCTION

Oil is essential for the global energy sector. The International Energy Agency (IEA) predicts a demand of 20 million barrels of oil per day by 2050. With the advancement of the petroleum industry in the extraction, transportation, storage, and refining, the risk of oil pollution has increased[1]. The chemical makeup of petroleum includes a complex combination of aromatic hydrocarbons, aliphatic hydrocarbons, heterocyclic hydrocarbons, asphaltenes, and non-hydrocarbon compounds. The aliphatics, aromatics, asphaltenes, and resins could be classified as general classes[1]. Brazil has intense oil exploration activity, occupying the ninth position in the world ranking of petroleum producers, with a volume of 1.1 billion barrels/year, of which 97.4% corresponds to offshore production[2]. Due to the high activity at sea, this region is more susceptible to accidents, such as oil spills, making it possible to contaminate coastal environments. In addition, the high toxicity, carcinogenicity, mutagenicity, and teratogenicity of petroleum pollutants predominantly affect the entire human food chain[1]. In this scenario, sustainable techniques to circumvent spills are extremely important in the Brazilian context.

Around 60-90% of the chemical composition of petroleum is biodegradable[1], so bioremediation has proven to be a promising degradation technique. *Yarrowia lipolytica* has emerged as a promising agent in the bioconversion of oils and fats due to its ability to utilize hydrophobic substrates as a carbon source efficiently[3]. In this context, biosurfactants emerge as a relevant class of compounds produced by microorganisms. They are amphipathic molecules capable of reducing surface tension with numerous industrial applications[4].

The aliphatic fraction constitute a significant group in crude oil, and their exclusion from oil-contaminated fields has become an ecological concern and is considered beneficial for improving recovery. Aromatics present in crude oil, with or without alkyl substituents, and their fractions are considered the second major group after the aliphatic fraction in crude oil. They are reported to be potentially dangerous as some of them are highly mutagenic or carcinogenic. Both the resin and asphaltene fractions contain polar non-hydrocarbon chemicals in contrast to the aliphatic and aromatic fractions. The former is mostly composed of carbon and hydrogen, with traces of nitrogen, sulfur, and oxygen. Asphaltenes are high molecular weight chemicals that are insoluble in solvents like n-heptane[1].

Due to their extremely complex structure, asphaltenes have very condensed carbon chains that are difficult for microorganisms to access, making their bioconversion less viable[1]. Due to this, an asphaltene-free fraction was tested. The present study aims to evaluate the biodegradation potential of a strain of *Yarrowia lipolytica* IMUFRJ 50682 isolated from Guanabara Bay applied to convert a fraction of asphaltene-free crude oil into biosurfactant using corn maceration liquor in the culture medium.

2 MATERIAL & METHODS

The wild-type strain *Yarrowia lipolytica* 583 IMUFRJ 50682 selected from an estuary of Guanabara Bay in Rio de Janeiro, Brazil[5] was maintained in YPD-agar medium (yeast extract 1% w/v, peptone 2% w/v, glucose 2% w/v, agar 3% w/v). The cells were used to inoculate 200 mL of YPD medium (yeast extract 1% w/v, peptone 2% w/v and glucose 2% w/v) in 500 mL Erlenmeyer flasks for the pre-inoculum. Pre-inoculum was cultivated at 160 rpm, 28 °C for 72 h.

The experiments were carried out in 500 mL Erlenmeyers containing 200 mL of mineral medium, 1% w/v (2g) of corn steep liquor, 1% v/v (2mL) of the crude oil asphaltene-free fraction (fraction + limonene) and an initial cell concentration of approximately 1 g/L

(from pre-inoculum). Control experiments, without the asphaltene-free fraction, were carried out for comparison purposes. The experiments were conducted for 264 hours, at 28° C and 160 rpm. Samples were taken after 0, 48, 96, 168, 216 and 264 h to analyze microbial growth and emulsification index. Mineral medium was composed of (in g/L): 7 KH_2PO_4, 2.5 Na_2HPO_4, 1.5 $MgSO_4.7H_2O$, 0.2 $CaCl_2.2H_2O$, 0.15 $FeCl_3.6H_2O$, 0.02 $ZnSO_4.7H_2O$ and 0.06 $MnSO_4.H_2O$. Corn steep liquor was obtained from Ingredion as a waste material.

The asphaltene-free fraction acquirement from crude oil was by cold extraction was carried out with the solvent D-limonene according to the methodology proposed by Ferreira et al.[6]. The product obtained was a solution of asphaltene-free crude oil diluted in a 1:8 ratio of D-Limonene.

Cell concentration was measured by absorbance at 570 nm and correlated with dry weight of cells using a standard curve.

The emulsification index was determined by vortexing for 2 minutes a mixture of 1 mL of cell-free medium and 1 mL of hexadecane. After 24 hours, the emulsification index was calculated by the ratio of the emulsion height measurement to the total mixture height, times 100.

3 RESULTS & DISCUSSION

Limonene is considered a green solvent with low toxicity, which is the reason it was chosen as a solvent in this study. For verification of the low toxicity and the behavior of *Y. lipolytica* in this solvent, the tests were carried out using growth media with limonene as a negative control. Figure 1 shows the mean cell concentration replicates experiments. The medium containing the crude oil asphaltene-free fraction presented higher cell concentration when compared to the control experiment, suggesting that the yeast used this crude oil fraction as a carbon source.

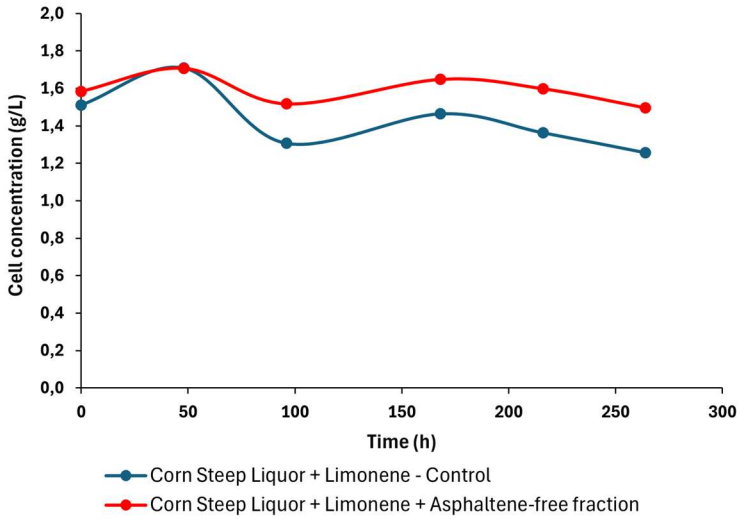

Figure 1 Cell growth profiles from *Yarrowia lipolytica* cultivation Erlenmeyer flasks (500 mL) containing 200 mL of media with corn steep liquor and limonene, with and without the crude oil asphalthene-free fraction, at 160 rpm, 28 °C.

Figure 2 represents the average emulsification index from replicates, showing that *Y. lipolytica* was able to produce biosurfactant in both media. For the medium with corn steep liquor and limonene (the solvent used in the extraction of asphatene), greater productivity is observed since EI is detected (30%) since 48 h. However, medium with crude oil asphaltene-free fraction showed a higher IE value (45.2%) at the end of the process.

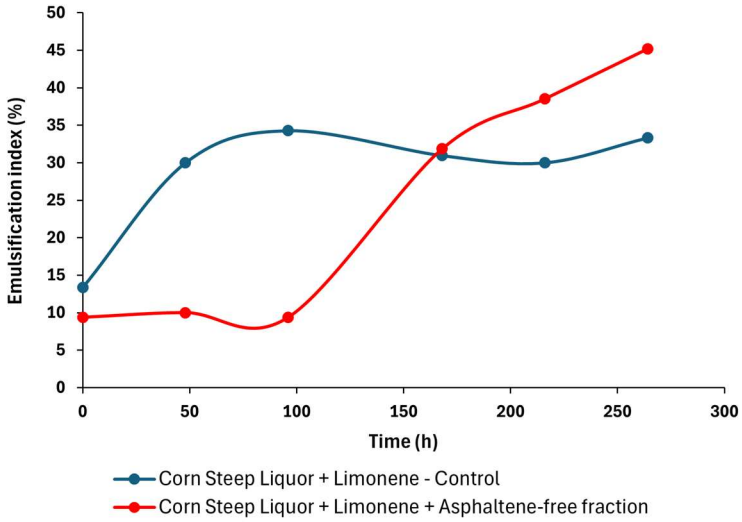

Figure 2 Emulsification index from *Yarrowia lipolytica* cultivation Erlenmeyer flasks (500 mL) containing 200 mL of media with corn steep liquor and limonene, with and without crude oil asphalthene-free fraction, at 160 rpm, 28 °C.

4 CONCLUSION

Yarrowia lipolytica was able to maintain cell growth in media containing crude oil asphaltene-free fraction with corn steep liquor. This strain produced biosurfactant with corn steep liquor and limonene, in the presence or absence of the crude oil fraction. This results shows the potential to use *Y. lipolytica* in the biodegradation of crude oils fraction to produce biomolecules.

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