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Potential of filamentous fungi in the biodegradation of petroderivatives and evaluation of the effect of surfactants

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ABSTRACT
The contamination of the environment by petroleum products is a current and growing concern due to high toxicity and difficult removal. New ecologically viable technologies, such as bioremediation, have been gaining prominence for using microorganisms and their metabolites to remove or degrade pollutants that affect marine and terrestrial ecosystems. In this context, the objective of this work was to evaluate the growth potential of filamentous fungi (Aspergillus foetidus and Rhizopus arrhizus) in petroderivatives, thus evaluating the potential of fungi in the biodegradation of petroderivatives (burnt motor oil, kerosene and diesel oil), in the presence and absence of surfactants. Therefore, the microorganisms were submitted to acclimatization in Sabouraud solid medium supplemented with different concentrations of burned motor oil (0%, 3% and 5%). Growth occurred during 72h at 28ºC. Then, the microorganisms were transferred to tubes containing Haas Bushnel medium and the 2,6-dichlorophenol-indophenol redox indicator (DCPIP) in the presence and absence of surfactants (natural and chemical). The results showed that Aspergillus foetidus and Rizopus arrhizus were able to grow at the maximum concentration of the petroderivative (5%), being therefore selected for the study of biodegradation. Aspergillus foetidus acclimatized with 5% of the burned engine oil was able to degrade (100%) the kerosene, both in the presence and absence of surfactants (chemical and natural). For the biodegradation of burnt engine oil (OQM) by Aspergillus foetidus the natural surfactant acted as a facilitating agent of biodegradation resulting in 100% degradation. Rhizopus arrhizus showed excellent biodegradation potential of diesel oil (100%), both in the presence and absence of surfactants (chemical and natural). Therefore, Aspergillus foetidus and Rhizopus arrhizus are promising microorganisms for biodegradation applications of kerosene and diesel oil, respectively. In addition, it was possible to prove that the surfactants (chemical and natural) did not show significant influence on the biodegradation of petroderivatives.

Keywords: Filamentous Fungi, Biodegradation, Surfactants, DCPIP.

1 INTRODUCTION

The pollution of the environment by petroderivatives resulting from accidental leaks or spills, use of by-products and the improper disposal of these compounds occur frequently in the world (ORTEGA et al., 2018; KUMAR et al., 2021).

Petroleum corresponds to a complex mixture of various hydrocarbons that has high viscosity, high density and low fluidity (SHI et al., 2021). Studies show that as the amount of benzene rings increase in petroleum hydrocarbons, the more difficult they become to degrade, becoming a major problem due to their low compatibility with the environment (low degradability) and their toxicological potential (JIMOH AND LIN, 2019; AMBUST et al., 2021; SARUBBO et al., 2022).

In refining, petroleum is fractionated into various types of materials, such as naphtha, gasoline, kerosene, lubricating oils, etc. (DA SILVA, 2022). Diesel oil is one of the most common petroleum products obtained from the refining of crude naphtha. It included high concentrations of hydrocarbons and low concentrations of sulfur, nitrogen and oxygen. flammable, toxic, low volatility product, with a strong smell (COSTA, 2022), while kerosene is composed of paraffins, cycloparaffins, aromatics and oleofins, with carbon chains ranging from C9 to C20, being considered a toxic pollutant for human health and the environment (SILVA, 2023).

Physical and chemical agents are commonly applied to assist in the process of controlling and reducing environmental pollution caused by petroderivatives. However, due to the negative consequences of these treatments, it is necessary to develop new technologies that are biocompatible for recovery, removal or degradation of oil and derivatives (LAWNICZAK et al., 2020).

An ecologically friendly and efficient alternative is the use of microorganisms for the transformation of petroleum hydrocarbons into biomolecules of high added value (WU et al., 2019; SUBASHCHANDRABOSE et al., 2019; ZEGZOUTI et al., 2020). Allied to this biological alternative, studies reveal that filamentous fungi and yeasts can use petroleum hydrocarbons as a carbon source for growth, resulting in the biodegradation of these pollutants (BERDE, et al., 2022).

Microorganisms previously acclimatized in hydrophobic compounds (diesel oil, petroleum, kerosene, etc.) are indicated as promising for degradation of petroderivatives by the natural physiological conditions of adaptation to the contaminant (PASSOS et al., 2009).

Studies report the importance of adding a surfactant for the enhancement of this biodegradation process, as they can increase the solubility of pollutants (fungi and bacteria) (ABENA et al., 2019; Almansoory et al., 2019).

Therefore, the objective of this work was to investigate the potential of *Aspergillus foetidus and* Rhizopus arrhizus *grown in petroderivatives, as well as to identify the potential for biodegradation in the presence of chemical and natural surfactant.*

2 METHODOLOGY

2.1 MICROORGANISMS

The microorganisms used were *Aspergillus foetidus UCP 360 and* Rhizopus arrhizus *UCP 1607 deposited in the culture bank of the Center for Research in Environmental Sciences and Biotechnology*

(NPCIAMB) of the Catholic University of Pernambuco, Recife – Brazil, registered in the World Federation for Culture Collections (WFCC).

2.2 HAAS BUSHNEL HALF

The Bushnel Haas – BH medium has the following composition: 0.2 g/L MgSO4, 0.02 g/L CaCl $_2$, 1.0 g/L KH2PO4, 1 g/L K2HPO4, 1.0 g/L NH4NO3 and 0.05 g/L FeCl2. This medium was previously sterilized at 121ºC for 15 minutes and used for biodegradation studies.

2.3 REDOX INDICATOR 2,6-DICHLOROPHENOL-INDOPHENOL-DCPIP

The redox indicator 2,6-dichlorophenol-indophenol (DCPIP) was used to detect the degradation potential of petroderivatives by microorganisms. The method considers positive for biodegradation when the DCPIP changes the original color from blue (oxidized state) to colorless (reduced state) (HANSON et al., 1993).

2.4 PETRODERIVATIVES

The petroderivatives used for the biodegradability test were burnt engine oil (OMQ) collected at a local fuel station, previously sterilized at 100ºC, diesel and kerosene (commercial) sterilized by filtration in a membrane of 0.22 μm porosity (KASVI, model K18-230).

2.5 SURFACTANTS

The natural surfactant (biosurfactant) used for the study of biodegradation was produced by the fungus *Cunninghamella echinulata UCP* 1299 (ANDRADE et al., 2018) isolated from the soil of the Caatinga in Pernambuco, Brazil, obtained from the UCP Culture Collection, Catholic University of Pernambuco, registered in the World Federation for Culture Collections (WFCC). The chemical surfactant used was Tween-80 solubilized at 1%.

2.6 ACCLIMATIZATION OF MICROORGANISMS

The microorganisms were grown in solid sabouraud medium containing burnt motor oil – OMQ. For this method, OMQ was added in different concentrations (0%, 3% and 5%) in Petri dishes containing Sabouraud Agar medium. The experiment was carried out in triplicate. Soon after, the plates were inoculated with the respective microorganisms for acclimatization at 28ºC for 72h.

2.7 INOCULUM

After the growth of the acclimatized microorganisms in Sabouraud medium containing concentrations of 0%, 3% and 5% of the petroderivative (burnt motor oil), a disc of the fungal mycelium (6 mm) was used as inoculum in the biodegradation assay.

2.8 STUDY OF THE BIODEGRADATION OF PETRODERIVATIVES

The biodegradation potential of petroderivatives (burnt engine oil, kerosene and diesel) by microorganisms was investigated in test tubes containing 9.7 mL of Bushnel Has medium – BH. Soon after, 200 µL of 2,6-dichlorophenol-indophenol – DCPIP redox indicator solution (0.5 g/L), 100 µL of the petroderivative, 100 µL of the natural surfactant, 100 µL of the chemical surfactant and the inoculum were added to the BH medium according to the conditions described in Table 1. The control media were prepared under the same conditions in the absence of the inoculum. The result of the degradation potential of petroderivatives was analyzed from the reduction of the DCPIP indicator (HANSON et al., 1993). The test tubes were incubated under static conditions and in the absence of light for 72h at 28ºC.

Table 1: Conditions for evaluation of biodegradation using different petroderivatives (burnt motor oil, kerosene and diesel)

PETRODERIVATIVE 1- BURNT ENGINE OIL						
Conditions	**Half BH (µL)**	**DCPIP (µL)**	**Inoculum (Disc)**	**Surfactant natural (µL)**	**Chemical surfactant (µL)**	**Petrode-rivado (µL)**
1	9,7	200	-	-	-	100
2	9,7	200	1	-	-	100
3	9,7	200	1	100	-	100
4	9,7	200	1	-	100	100
PETRODERIVATIVE 2- KEROSENE						
5	9,7	200	-	-	-	100
6	9,7	200	1	-	-	100
7	9,7	200	1	100	-	100
8	9,7	200	1	-	100	100
PETRODERIVADO 3- DIESEL						
9	9,7	200	-	-	-	100
10	9,7	200	1	-	-	100
11	9,7	200	1	100	-	100
12	9,7	200	1	-	100	100

* (-) Absence of the component.
Source: The author.

2.9 DETECTION OF BIODEGRADATION

To identify the occurrence of biodegradation of petroderivatives (burnt motor oil, kerosene and diesel) by microorganisms, aliquots of 2 mL were removed from each tube, transferred to eppendorfs and subjected to centrifugation at 12000 rpm and 15ºC for 5 min. Soon after, absorbance at 600 nm was determined in a UV/Vis spectrophotometer (model Libra S32, Biochrom Ltd.) (Ozyurek & Bilkay, 2020). The absorbance data collected were used to calculate the percentage of biodegradation according to Equation 1 (MAMITHA, et al., 2013).

(Eq 1)

$$\text{Biodegradation \%} = \left(1 - \frac{sample\ absorbance}{control\ absorbance}\right) x\ 100$$

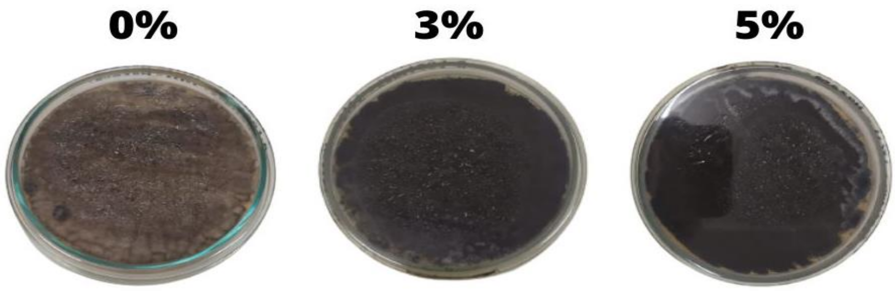
3 RESULTS AND DISCUSSION

3.1 GROWTH POTENTIAL OF FILAMENTOUS FUNGI ACCLIMATIZED IN BURNT MOTOR OIL

The fungi *Aspergillus foetidus (Figures 1) and* Rizopus arrhizus (Figures *2*) were able to grow in Saboraud agar medium supplemented with burnt motor oil at all tested concentrations (0%, 3% and 5%) after 72h (Figure 2). Thus, *Aspergillus foetidus* and *Rizopus arrhizus*, grown at the maximum concentration of the petroderivative (5%), were selected for the biodegradation experiments.

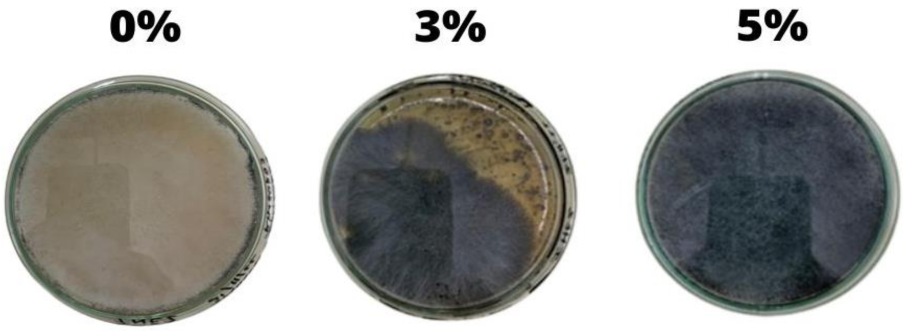
Several authors point out that the previous adaptation of microbial cultures to the pollutant is an essential step in the process of biodegradation of hydrocarbons, resulting in a significant increase in the degradation potential (AAMAND, JORGENSEN, ARVIN and JENSEN, 1989; LEAHY and COLWELL, 1990; Sálleh et al., 2003; MIRANDA et al., 2007; ALMEIDA, 2013; COSTA et al., 2022).

Figure 1: Acclimatization *of Aspergillus foetidus* in different concentrations of burned motor oil: 0%, 3% and 5%



Source: The Author, 2023.

Figure 2: Acclimatization *of Rhizopus arrhizus* in different concentrations of burned motor oil: 0%, 3% and 5%



Source: The Author, 2023.

3.2 POTENTIAL OF *ASPERGILLUS FOETIDUS* IN THE BIODEGRADATION OF PETRODERIVATIVES AND STUDY OF THE INFLUENCE OF NATURAL SURFACTANT AND CHEMICAL SURFACTANT

Aspergillus foetidus UCP 360 acclimatized in 5% burnt engine oil is a promising biodegradation fungus, promoting 93-100% degradation. Among the petroderivatives tested, kerosene was the one that *A. foetidus* managed to fully degrade (100%). The surfactants (natural and chemical) did not influence the acceleration of the degradation of kerosene and diesel oil. In the process of biodegradation of burnt motor oil (OQM) by *Aspergillus foetidus* there was a significant influence of the natural surfactant as a facilitating agent of biodegradation resulting in 100% degradation (Table 2). Da Silva et al. (2022) obtained similar results in the growth of *Aspergillus* sp. inoculated in Saboraud agar medium supplemented with burnt motor oil and after 72h there was excellent microbial growth at the maximum concentration of petroderivative tested (10% OMQ).

Table 2: Evaluation of the potential of *Aspergillus foetidus* UCP 360 (acclimatized in 5% of burned engine oil) in the biodegradation of petroderivatives and influence of natural and chemical surfactant

***Tests**	**Composition**	**Acclimatization concentration of *Aspergillus foetidus* (%)**	**Petroderivados**		
			OMQ (%)	**Kerosene (%)**	**Diesel (%)**
2,6,10	medium BH + DCPIP + inoculum	5	93,3	100	93,0
3, 7, 11	medium BH + DCPIP + inoculum + natural surfactant + petroderivative	5	100	100	93,3
4, 8, 12	medium BH + DCPIP + inoculum + chemical surfactant + petroderivative	5	96,6	100	93,3

* Composition of the tests - Test 2: medium BH + DCPIP + inoculum + OMQ; Assay 3: BH medium + DCPIP + inoculum + biosurfactant + OMQ; Assay 4: BH medium + DCPIP + inoculum + chemical surfactant + OMQ; Test 6: BH + DCPIP medium + inoculum + kerosene; Assay 7: BH medium + DCPIP + inoculum + biosurfactant + kerosene; Test 8: BH medium + DCPIP + inoculum + chemical surfactant + kerosene; Test 10: medium BH + DCPIP + inoculum + diesel; Test 11: medium BH + DCPIP + inoculum + biosurfactant + diesel; Test 12: medium BH + DCPIP + inoculum + chemical surfactant + diesel.

3.3 POTENTIAL OF *RHIZOPUS ARRHIZUS* UCP 1607 IN THE BIODEGRADATION OF PETRODERIVATIVES AND STUDY OF THE INFLUENCE OF NATURAL SURFACTANT AND CHEMICAL SURFACTANT

Rhizopus arrhizus UCP 1607 showed excellent biodegradation potential of diesel oil (100%). In addition, *R. arrhizus* was also potentially able to degrade burnt engine oil and kerosene with values between 93-96% biodegradation. On the other hand, the surfactants (natural and chemical) did not influence the acceleration of the degradation of kerosene and burnt engine oil (Table 3). Thus, it was possible to verify that diesel oil was an excellent source of carbon and energy for *Rhizopus arrhizus*.

Table 3: Evaluation of the potential of *Rhizopus arrhizus* UCP 1607 in the biodegradation of petroderivatives

***Tests**	**Composition**	**Acclimatization concentration of *Rhizopus arrhizus* (%)**	**Petroderivados**		
			OMQ (%)	**Kerosene** (%)	**Diesel** (%)
2,6,10	Medium BH + DCPIP + inoculum	**5**	96,6	100	100
3, 7, 11	BH medium + DCPIP + inoculum + natural surfactant + petroderivative	**5**	96,6	96,6	100
4, 8, 12	BH medium + DCPIP + inoculum + chemical surfactant + petroderivative	**5**	93,3	96,6	100

* Composition of the tests - Test 2: medium BH + DCPIP + inoculum + OMQ; Assay 3: BH medium + DCPIP + inoculum + biosurfactant + OMQ; Assay 4: BH medium + DCPIP + inoculum + chemical surfactant + OMQ; Test 6: BH + DCPIP medium + inoculum + kerosene; Assay 7: BH medium + DCPIP + inoculum + biosurfactant + kerosene; Test 8: BH medium + DCPIP + inoculum + chemical surfactant + kerosene; Test 10: medium BH + DCPIP + inoculum + diesel; Test 11: medium BH + DCPIP + inoculum + biosurfactant + diesel; Test 12: medium BH + DCPIP + inoculum + chemical surfactant + diesel.

Source: Author.

4 CONCLUSION

Aspergillus foetidus and *Rizopus arrhizus* were able to grow at the maximum concentration of the petroderivative (5%). The maximum biodegradation by *Aspergillus foetidus* occurred when using kerosene as a carbon source, while for *Rhizopus arrhizus* the maximum biodegradation potential was when using diesel oil (100%), which proves that these filamentous fungi have potential for use in environments impacted with petrodrivados. Additionally, the presence of the surfactant (chemical and natural) did not influence biodegradation.

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