VEGETABLE OIL BIODEGRADATION BY FILAMENTOUS FUNGI

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ABSTRACT

The rapid progression of technology has undeniably revolutionized the way of communicate and travel. However, this has also led to environmental deterioration, especially through the improper disposal of cooking oil. This research aims to utilize filamentous fungi for the biodegradation of cooking oil. In the experimental phase, four different filamentous fungi were tested using a synthetic medium. *Penicillium crysogenum*, *Penicillium aculeatus*, and *Aspergillus. niger* exhibited substantial enzymatic production. Particularly, *A. niger* and *P. aculeatus* successfully generated the crucial oil-degrading enzyme, with measured values of approximately 0.6 $g.L^{-1}$ and 0.4 $g.L^{-1,}$ respectively. The growth curves of the fungi were analyzed, revealing that *P. aculeatus* displayed the most favorable growth results, reaching approximately 0.38 $g.L^{-1}$.

Keywords: Biodegradation of cooking oil. Filamentous Fungi. *P. crustosum. P. chrysogenum*

1 INTRODUCTION

Technological advancement has brought numerous benefits, such as improved communication, transportation, interactivity, and access to resources for exploration. However, a major environmental issue that has garnered significant attention is the improper disposal of solid waste, with oil waste from cooking activities being a major contributor. When this waste is carelessly discarded, it can contaminate water sources, releasing up to 200 million liters of oil into rivers and lakes, thus seriously harming the environment [1].

However, despite the awareness of proper disposal practices, many individuals continue to inappropriately dispose of oil waste, causing significant damage to the environment. Improper disposal methods, such as pouring oil down drains or sewage networks, can lead to the waterproofing of beds and soil, exacerbating numerous environmental problems [2]. An effective method for addressing vegetable oil contamination in effluents, especially when the oil is no longer reusable upon contact with water, is through biodegradation using filamentous fungi. These microorganisms possess substantial potential for biodegradation due to their production of biodegradable enzymes, such as lipases. These fungi utilize pollutants like cooking oil as a source of carbon and energy [3].

Fungi are renowned for their natural role in breaking down complex molecules such as lignin and cellulose [3]. Costa [4] utilized fungi species *Penicillium crustosum* and *Penicillium crysogenum* to effectively biodegrade Linear Alkylbenzene Sulfonate Sodium, achieving removal rates of up to 99.5%. Similarly, Santos [5] employed *P. crysogenum* in aerobic submerged cultures, resulting in a high level of LAS biodegradation at 99.4%, significantly outperforming conventional anaerobic treatments. Gontijo [6] also utilized these microorganisms and obtained remarkable results in the biodegradation of Linear Alkylbenzene Sodium Sulfonate. *P. crustosum* demonstrated a higher biodegradation rate, reaching 98.7%, while *P. chrysogenum* achieved a maximum biodegradation rate of 96%, with a 2.7% difference between the two fungi. In this context, the present study aims to use filamentous fungi in bench experiments for the biodegradation of vegetable oils.

2 MATERIAL & METHODS

The fungi selected for reactivation were chosen based on their ability to produce lipase, the enzyme necessary for the biodegradation of oil. The experiments were conducted with the fungi *Penicillium crysogenum*, *Penicillium crustosum*, *Aspergillus aculeatus*, and *Aspergillus niger*, which were isolated from the effluent of the textile industry in the municipality of Divinópolis - MG and later identified [1]. The microorganisms were immersed in mineral oil for storage and conservation of the strains. At this stage, the fungi were placed in Petri dishes, in a culture medium using Potato dextrose agar at 39 $g.L^{-1}$, so that they were reactivated for growth and reproduction. The cryopreserved fungi were inoculated into the plates and incubated at 25ºC until the growth of the fungus and spore production.

To determine whether the microorganisms produced the enzyme lipase, the Tween® 80 medium method was employed. According to Gontijo [6], to identify strains that can hydrolyze ester bonds through lipolytic activity, the microorganisms were inoculated into Petri dishes containing Tween® 80 medium as a substrate. The culture medium consisted of ($g.L^{-1}$): 10.0 peptone; 5.0 NaCl; 0.1 $CaCl_2.2H_2O$; 10.0 of Tween® 80, and finally, 20.0 agar, with a pH of 7.0. Subsequently, the lipolytic activity was indicated by a visible residue, namely, an opaque halo around the colony. In the respective Petri dishes, 10 µL of the fungus spore solution was placed, and then it took approximately 5 to 7 days to analyze the halos formed in the plates.

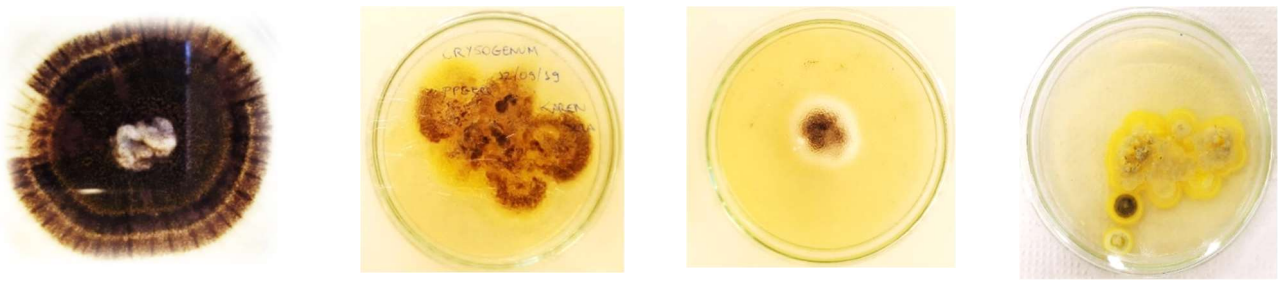
To evaluate the behavior of the fungus in the applied medium, the growth kinetics were assessed over five days, with the daily result representing the mean triplicate value. The concentrations of the reagents used were as follows ($g.L^{-1}$): $C_6H_{12}O_6$ (10.0);

$MgSO_4$ (1.1); $(NH_4)_2SO_4$ (1.0); KH_2PO_4 (5.44); K_2HPO_4 (6.98); and $CuSO_4$ (0.01). Erlenmeyer flasks were labeled and incubated on an orbital shaker at 180 rpm and 25°C. Following the same procedures, vegetable oil (0.35µL) was used as a substrate to determine which medium the fungi would produce more biomass. In this stage, the fungi *A. aculeatus*, *P. crysogenum*, and *A. niger* were used. The amount of oil used was analyzed and calculated according to Conama Resolution No. 430, May 13, 2011. For the experiment, the vegetable oil used was soybean oil from the Corcovado brand, containing refined soybean oil (Genetically modified from Agrobacterium tumefaciens/Bacillus thuringiensis) and Citric Acid Antioxidant.

In the biodegradation stage, fungi that exhibited better growth conditions were selected using the growth curve method. For the biodegradation process, the components used included vegetable oil and an emulsifier, in this case, Tween® 80. This non-anionic surfactant facilitates the connection between the oil, as demonstrated in other studies, for example, as described in Gontijo [6].

3 RESULTS & DISCUSSION

After 7 days of growth, it was observed (Figure 1) that the fungus exhibiting the best growth results on the plate was the microorganism *Aspergillus aculeatus* (Figure 1a). A vast carpet was visible, indicating a significant number of spores. The fungus with the second highest growth was *P. crysogenum*, showing a small carpet with a few greenish regions on the plate and containing a satisfactory number of spores (Figure 1b). As for the microorganism *A. niger*, there was a small growth, forming a small green circle on the plate, and the number of spores generated was minimal (Figure 1c). Finally, upon analyzing the plate with the microorganism *P. crustosum*, an exceptionally low growth was observed, with the number of spores harvested not being sufficient to continue the process (Figure 1d).



(a) (b) (c) (d)

Figure 1: Reactivation of fungi: A) *A. aculeatus*, B) *P. crysogenum*, C) *A. niger,* and D) *P. crustosum*. Source: Personal, 2020.

After six days of the experiment, a ruler was used to measure the halo formed in some of the plates. The measurement results can be seen in Table 1, indicating promising results for three fungi.

Table 1: Measurements of the halo formed from each fungus for 6 days.

Fungi	1st day	6th day	Spores (mL^{-1})
P. crysogenum	0,0 cm	4,0 cm	11,8 $x10^7$
P crustosum	0,0 cm	0,0 cm	-
A. aculeatus	0,0 cm	5,5 cm	5.3 $x10^7$
A. niger	0,0 cm	4,4 cm	4.0 $x10^7$

From the data provided in Table 1, it was observed that after six days, the fungus that produced the most significant amount of lipase was *A. aculeatus*, as it formed a larger halo compared to the other fungi. *P. crysogenum* and *A. niger* also formed halos with satisfactory results. Conversely, after six days, it was noted that *P. crustosum* did not form a halo, indicating that the microorganism does not produce the enzyme necessary for the degradation of vegetable oil, and therefore it will not be used in the experiment. Following the calculations based on the data in Table 1, 3.930 µL of soybean oil, 100 µL of fungus, and 70 mL of the medium were added to each Erlenmeyer flask for the experiments. Subsequently, the growth curve of the fungus was plotted in a medium with and without oil for comparison, as shown in Figures 2 and 3.

Figure 2 shows that the fungus that showed a result of cell growth was the microorganism *A. aculeatus*. Note that between days 2 and 3, there was a growth of around 0.25g. From day 3 to day 5, there was an increase in growth reaching values around 0.7g. Based on the analysis obtained, it was determined that the best microorganisms to be used in the experiment are *A. niger* and *A. aculeatus*, as they exhibited greater production of dry mass compared to the other tested fungi. Comparing these results with Costa [6], it was noted that in this study the microorganism *A. aculeatus* exhibited the second-highest growth during the 5 days of growth kinetics. Drawing a parallel between the two studies, under similar conditions, the microorganism *A. aculeatus* showed the best fungal growth, whereas, in Costa's study, this particular fungus needed 24 hours to adapt to the environment.

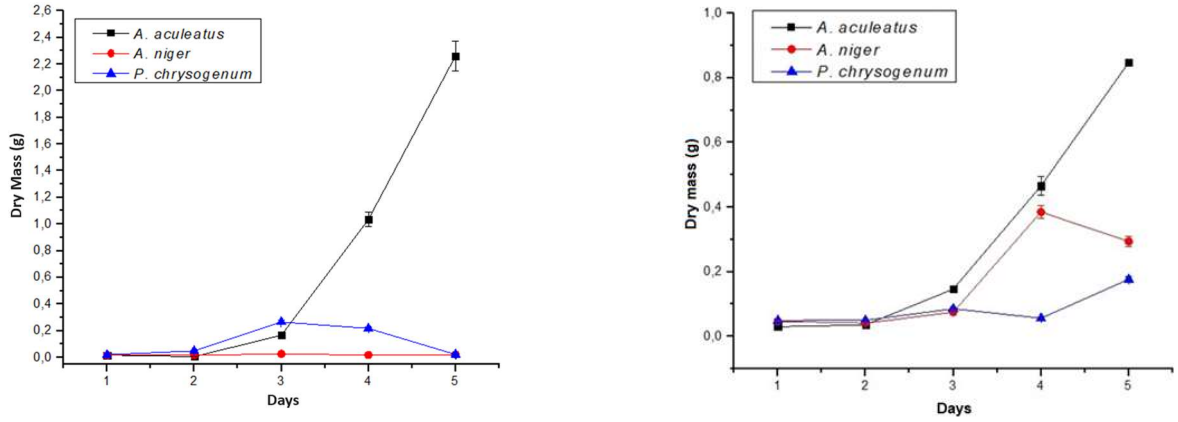


Figure 2: (a) Growth curve of filamentous fungi without oil; (b) Growth curve of filamentous fungi with oil.

The samples containing oil and Tween® 80 were analyzed for 5 days. It was observed that the microorganism *A. aculeatus* showed greater growth during the five days, reaching values of approximately 0.38 g. However, on the fifth day, there was a small decrease in dry matter, with values dropping to approximately 0.3 g. This decrease may have occurred due to the reduction of available nutrients in the medium, specifically glucose and oil. In contrast, the microorganism *A. niger* exhibited higher dry mass growth on the third day, with values of 0.1 g. However, from the 4th day of the experiment, the fungus growth decreased, reaching values of approximately 0.038 g.

In this study, the fungus *A. aculeatus* showed the best results in dry mass production and lipase production. In other biodegradation experiments using filamentous fungi, it was observed in Costa [2] that *A. aculeatus* did not achieve significant biodegradation results due to the long adaptation period of *Aspergillus* to the medium containing the detergent (slow lag phase), leading to delayed glucose consumption compared to the rapid assimilation observed in fungi of the genus *Penicillium*. In other experiments, the microorganism *P. crysogenum* showed promising results in dry mass production and achieved approximately 90% biodegradation. Similarly, in Gontijo [6], significant results were observed for *P. crysogenum* in biodegradation experiments with a surfactant, resulting in fungal growth with mass values ranging from 0.233 g to 0.9214 g and approximately 80 to 90% biodegradation of the surfactant used. However, in the present work, *P. crysogenum* exhibited different behavior; in the medium containing oil, the microorganism showed limited growth, with results ranging from 0.489 g to 0.766 g. It can be concluded that *P. crysogenum* was inefficient in a medium containing oil.

4 CONCLUSION

Based on the results obtained, the microorganism *Aspergillus aculeatus* has demonstrated the greatest potential for biodegradation of vegetable oil. It exhibited good growth in all research conducted, especially in the production of the enzyme lipase in the medium-containing oil. In contrast, the growth of other fungi (*P. crysogenum*, *A. niger*, and *P. crustosum*) was not as satisfactory. The growth curve, both with the use of oil and without, showed a significant amount of dry mass production for *A. aculeatus* compared to the other fungi. This fungal growth is crucial for the production of enzymes and ultimately for the biodegradation of the oil.

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