Using *Aspergillus niger* as an effective substitute for *Aspergillus tubingensis* in the degradation of plastics

Group 01-51

Abstract

In recent years, the development of various methods to effectively degrade plastic waste has been widely researched. Some of these methods include chemical degradation, photodegradation and biodegradation. This study aims to observe and compare the effectiveness of 2 strains from the fungal family *Aspergillus* in the degradation of 3 of the most used plastics. The fungal strains and plastics used in this study include *Aspergillus niger, Aspergillus tubingensis,* low-density polyethylene, polypropylene and polyurethane. The fungus was allowed to degrade the plastics for 4 weeks, with the mass of the plastics individually measured using an electronic weighing scale before and after the experiment. The results proved that both fungi were able to degrade all plastics to a certain extent, however, *A. niger* was observed to be much more effective than *A. tubingensis* for each of the different conditions tested.

Introduction

Problem addressed

Plastic is an integral part of society. It serves many purposes, from the common plastic bag to the diverse range of plastic bottles, plastic has improved our lives tremendously. However, being a man-made polymer with inorganic bonds (Jeffery, 2016), plastic takes hundreds of years to degrade (Harris, 2010). Plastic waste generated by society today will still be around for generations to come, polluting the environment for future generations. The problem stumping researchers around the world is finding a sustainable method of dealing with this plastic waste and reverse or prevent further plastic pollution. A relatively new field that may hold the key to solving the plastic pollution problem is biodegradation using various microorganisms.

Current solutions and their limitations

Thermal degradation

According to Madorsky and Straus (1959), plastics such as polystyrene and polyethylene can be degraded via pyrolysis under high temperatures that range from 362°C and at 850° C. It was also observed that higher temperatures and greater pressures caused greater fragmentation within the plastics, which means more degradation. However, the byproducts of thermal degradation include toxic gases like dioxins, furans, mercury and carbon dioxide, which can be detrimental to the environment (Verma et al., 2016).

Chemical degradation

Polymers like low-density polyethylene and polystyrene were found to be able to be degraded under environments of nitrogen oxides and oxygen (Sen & Pifer, 1998). However, a large amount of energy is required to carry out the process and many polymers are resistant to chemical degradation. This means that extensive research has to be conducted to find more suitable chemicals to degrade each type of plastic.

Photodegradation

Photooxidative degradation of various materials, including polymers, occurs under the presence of UV radiation, breaking polymer chains and causing gradual embrittlement (Haddad & Yousif, 2013). However, photodegradation of plastics via UV radiation is a very slow process and takes a lot of time, which cannot match the 6.3 billion metric tonnes of plastic waste produced each year (Parker, 2018).

Biodegradation using various microorganisms

Widespread research has gone into using microorganisms for the degradation of plastics, as well as the discovery of degradation enzymes produced by these microorganisms (Shimao, 2001) Various microorganisms with the ability to degrade plastics have been isolated and identified. The enzymes produced from these microorganisms can be isolated and produced synthetically. However, a suitable microorganism or enzyme for widespread commercial use has not been identified yet. This could be due to microorganisms taking too long to degrade the plastic, or due to the microorganisms being commercially inaccessible due to its high price.

Proposed solution

Biodegradation of plastics using fungal strains *A. niger* and *A. tubingensis.*

The fungal family *Aspergillus* has been found to have many species that possess the ability to degrade plastics. This study aims to investigate 2 strains from this family, *A. niger* and *A. tubingensis. A. niger* was reported to be able to degrade polyethylene and polypropylene (Arutchelvi et al, 2007), and *A. tubingensis* was found to have the ability to degrade polyurethane (Khan et al, 2017). However, the discovery of polyurethane degradation ability of *A. tubingensis* was fairly recent (Anwar, 2019) and *A. tubingensis* is a relatively expensive strain of fungus to procure. On the other hand, *A. niger* has known to have plastic degradation plastic for many years and is much more commonly found (Sobel, 2014), possibly making it a much cheaper alternative. The possibility of using *A. niger* to replace A. tubingensis in the degradation of polyurethane, polypropylene and polyethylene was investigated in this study.

Objective and hypothesis

Objective

The objective of this study was to investigate the difference in the effectiveness of *A. niger* and *A. tubingensis* in the biodegradation of LDPE, polypropylene and polyurethane. This was to determine if the cheaper *A. niger* can be used as a suitable alternative for *A. tubingensis* to degrade large piles of plastic waste.

Hypothesis

The hypotheses tested are as follows:

1. *A. niger* is more effective in degrading low-density polyethylene than *A. tubingensis*.
2. *A. tubingensis* is more effective in degrading polypropylene than *A. niger*
3. *A. tubingensis* is more effective in degrading polyurethane than *A. niger*.

Materials and equipment used

Plastics

1. Polyurethane in the form of a sponge obtained from 3M.
2. Polypropylene in the form of corrugated plastic board obtained from Popular.
3. Low-density polyethylene (LDPE) in the form of plastic bags obtained from NES Packaging.

Fungi

1. *A. niger* from Carolina Biological Supply.
2. *A. tubingensis* of strain ATCC 66876 from American Type Culture Collection.

Others

Potato dextrose agar
Petri dish
Electronic weighing scale
Ethanol
Autoclave
Scalpel
incubator

Experimental procedures

Preparation of plastics

3 pieces of each plastic were used to investigate each type of fungus. The plastics used were cut up into small little pieces to put them onto the Petri dishes. The plastics weighed are 0.017g, 0.076g and 0.46g respectively. The plastics were sterilized by immersing the plastics in 70% ethanol for 2 minutes. After removing the ethanol, the plastics are rinsed in sterile deionised water thrice. This helps to kill any microorganisms present on the surface of the plastics.

Synthesis of agar solution

1.8g of dipotassium phosphate, 4g of ammonium chloride, 0.2g of dilute magnesium sulfate solution, 0.1g sodium chloride and 0.01g of dilute iron sulfate solution were mixed with 1 litre of water and the solution is used for the agar solution. The agar solution was then poured into the Petri dishes and solidifying agent added to the agar to solidify agar before adding the fungus. The agar solution used did not contain any carbohydrates as the main source of nutrients of the fungi should be from the plastics and not in the agar.

Addition of fungi to plastics

The fungi are put into the petri dish by cutting off parts of the agar from the petri dish containing the fungus using a scalpel. *A.niger* is added to each petri dish containing the substrates. The entire process is repeated for *A. tubingensis*. The Petri dishes are sealed with tape and left inside incubators for 2 weeks.

Results and discussion

Measurement of final vs initial mass

The plastics in the agar culture containing *A. niger* and *A. tubingensis* did not show much visible change. However, several patches of the fungus were seen growing around the plastic, and some of the fungi were seen to be growing from the spore stage. However, the plastics felt much softer, showing that there are physical differences due to the degradation of plastics by the different fungus.

Observations with polyethylene

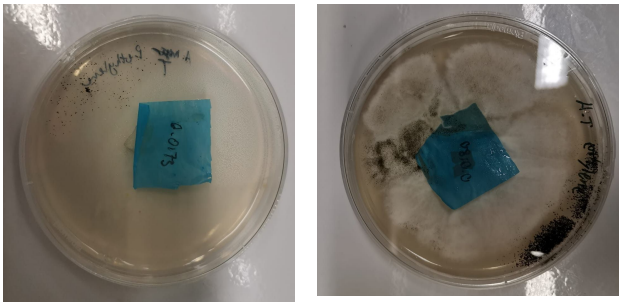
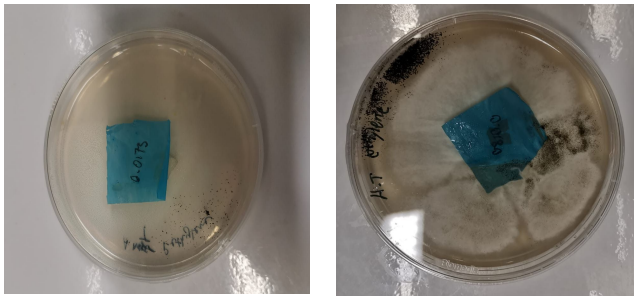


Fig 1.1: Images showing the before and after stages of growth of *A. tubingensis* on polyethylene

Fig 1.2: Images showing the before and after stages of growth of *A. niger* on polyethylene



As seen from both of the different cultures above, both fungi were capable of degrading polyethylene present in the different cultures after their incubation period of 2 weeks. It was observed that the polyethylene degraded by *A. niger* was much softer and easier to tear compared to the plastic degraded by *A. tubingensis*.

Observations with polypropylene

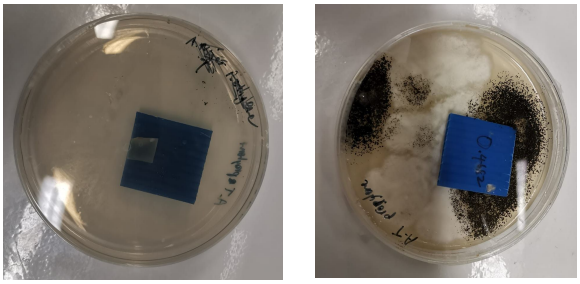
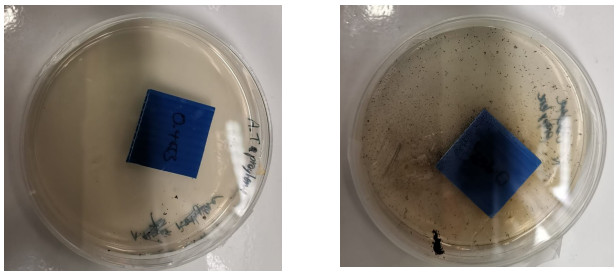


Fig 1.3: Images showing the before and after stages of growth of *A. tubingensis* on polypropylene

Fig 1.4: Images showing the before and after stages of growth of *A. niger* on polypropylene



As seen from the figures above, patches of each fungus were observed to be growing around the plastics, even being able to grow from the spore stage, showing that fungus was able to degrade the plastics for nutrients. Although no visible change is seen when the polypropylene board is exposed to the fungus, the board that was in contact with *A. niger* was much softer than the board exposed to *A. tubingensis*.

Observations with polyurethane

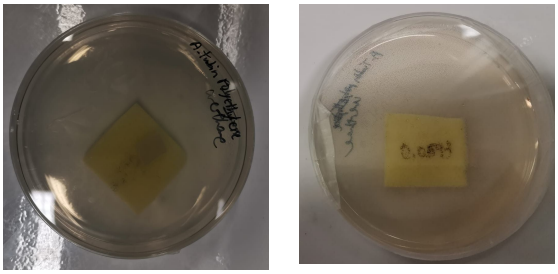
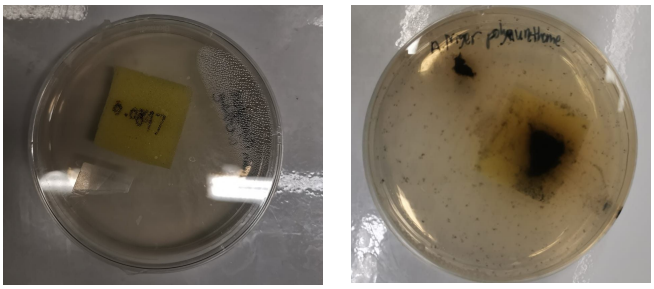


Fig 1.5: Images showing the before and after stages of the growth of *A. tubingensis* on polyurethane

Fig 1.6: Images showing the before and after stages of the growth of *A. niger* on polyurethane



As seen from the figures above, patches of fungi were observed to be growing around the plastics, showing that both fungi can degrade the plastics for nutrients for their growth. No visible change was observed, but the polyurethane that was exposed to *A. niger* was much softer and easier to tear than the plastic exposed to *A. tubingensis*.

An alternative method is also tested. This method allows the plastics to be fully exposed to the fungi in hopes that the degradation process can take place more rapidly and effectively. By putting the plastics in an agar broth containing the fungi, the fungi can degrade the plastics thoroughly. Visually, the plastic showed a great change in colour and parts of the plastic even broke off. The plastics all became much softer compared to the plastics that were put in Petri dishes.

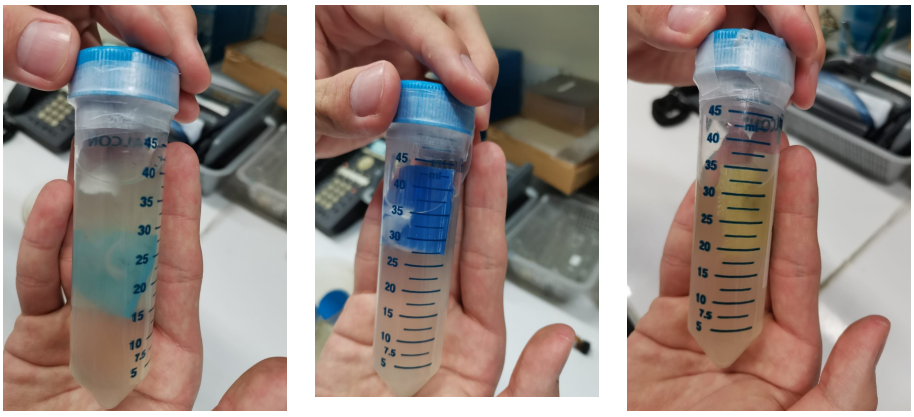


Fig 1.7: Images of the three types of plastics submerged in agar broth containing *A. niger*

Results

The initial and final masses of each piece of plastic was recorded and the decrease in mass and percentage decrease in mass was calculated.

The following graphs show the average change in mass of each type of plastic and fungi.

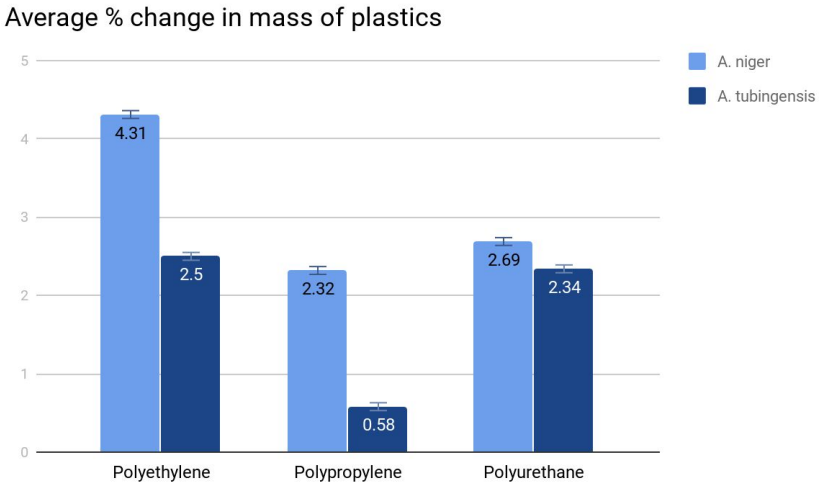


Fig 2: Graph showing the average percentage change in mass of plastics for *A. niger* and *A. tubingensis*

Comparison of the effectiveness of an alternative method

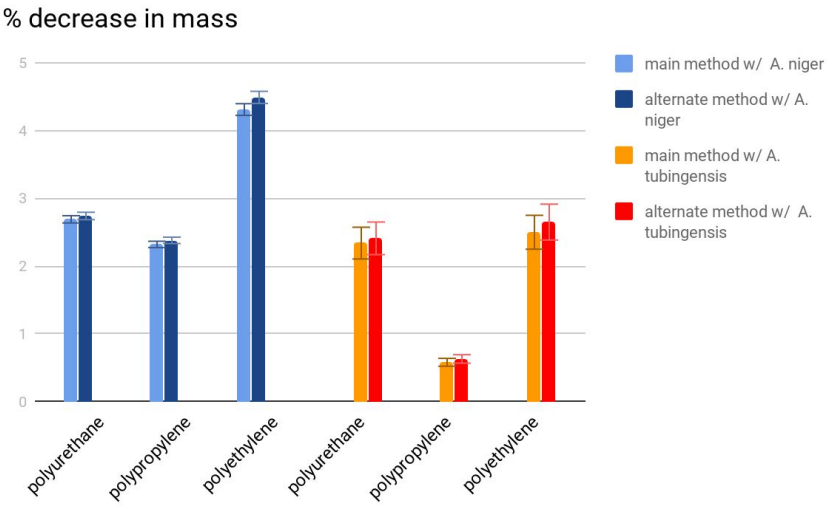


Fig 3: Graph showing the average percentage change in mass of plastics for *A. niger* and *A. tubingensis* using both the main method and the alternative method

As not enough replicates were performed, a statistical analysis could not be performed. However, visual inspection of the error bars which overlap suggests that the difference in the effectiveness of each method is not significant. However, both *A. tubingensis* and *A. niger* proved to be more effective at degrading all three types of plastics with the alternative method as compared to the main method used, suggesting that the alternative method may be more effective than the main method used.

Conclusion

From the difference in percentage change of *A. niger* and *A. tubingensis*, it can be concluded that *A. niger* is much more effective at degrading plastics than *A. tubingensis.* Since *A. niger* is the cheaper alternative, it can potentially be used in the widespread degradation of plastic waste. It can also be inferred from the results that polypropylene is the most resistant to degradation, followed by polyurethane and LDPE. It can also be seen that the alternative method that submerged the plastic in culture broth may be more effective than the initial method.

Future work

The rate or percentage of degradation over long periods of time can be investigated. There can also be further investigation into whether soaking plastics in fungal culture broth is the most ideal method for plastic degradation. There can also be additional experimentation on *A. niger* to find out if *A. niger* can degrade other plastics. There can also be further investigation into whether *A. niger* can be used in the widespread degradation of plastics, as well as more economical alternatives to *A. niger*.

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