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Evaluation of the enzymatic activity of some fungi isolated from plastic contaminated soils and their LDPE biodegradation ability

Marwah Sabeeh Altaee and Mustafa Abdul-ahab Al-Dossary
Basrah University, College of Science, Ecology Department
mustafa.najem@uobasrah.edu.iq

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Abstract

Thirteen fungal species were isolated from eight soil samples contaminated with plastic waste collected from several places in Basra governorate and tested for their enzymatic activity to three enzymes (manganese peroxidase, lignase and cellulase). This study has showed good enzymatic activity for the isolated fungi; 11 fungi were able to produce manganese peroxidase enzyme, nine fungi were able to produce lignase enzyme, 8 fungi produced cellulase enzyme, the fungus *Aspergillus niger* was selected to study its ability to biodegrade LDPE in liquid medium for 90 days. The result showed that this fungus was able to degrade 26.128% of the LDPE sheets after 90 days of incubation.

Key words: Fungi, Enzymatic activity, LDPE, Biodegradation.

Introduction

The enzymes secreted by fungi play an important role in the decomposition process of organic substances. There are different types of fungal enzymes involved in this process, such as manganese peroxidase, lignase and cellulase, etc. The degradation of all organic substances by fungi depends on the presence of these enzymes (Moreno *et al*., 2015). Manganese peroxidase enzyme is one of the most widespread enzymes that fungi can secrete; analyzing many harmful pollutants, including plastic pollutants, with high efficiency (Prasher and Chauhan, 2015).The lignin enzyme is a large molecule and has difficult decomposition properties. Lignin content in wood ranges from 18 to 30%, and it is highly complex due to its high molecular weight. Although this compound is difficult to decompose, some fungi, especially white rot fungi, were able to secrete a group of enzymes that can analyze this compound using lignase and manganese peroxidase (Knežević *et al*., 2014). In addition, cellulase enzyme is a group of enzymes that can break down cellulose, one of the most abundant organic compounds on earth with around 15-60 % of the cell wall components, into simple sugar components glucose (Karmakar and Ray, 2011).

These enzymes make fungi able to degrade a wide range of pollutants in the environment, including low-density polyethylene (LDPE), which is widely used as plastic compounds, accounting for about 64% of the total industrial plastic production because it is inexpensive and

flexible (Ndahebwa *et al*.,2018). Low-density polyethylene can be subjected to biodegradation by fungi as it can degrade effectively. These fungi are able to adapt to plastic pollutants and remove them completely without harming the environment comparison with the hazards of chemical methods or converting them to another form that is less harmful to the environment because they have an effective enzyme system consuming carbon as a source of energy (Usha *et al*., 2011; Kumar *et al*., 2013)

This study aimed to study the enzymatic activity of some fungi isolated from the contaminated area and select the pest species to study its ability to degrade the (LDPE) compound.

Material and methods

Fungal isolation

Eight soil samples were collected from different plastic dumping areas contaminated with plastic waste in Basra governorate. Fungi were isolated from soil samples by using the dilution method (Wicklow and Wittingham, 1974). Mineral salts agar (MSA) supplemented with low-density polyethylene granules was used for fungal isolation, the composition of the culture media (g/l) was: 0.5 K_2HPO_4, 0.04 KH_2PO_4, 0.1 NaCl, 0.002 $CaC1_2.H_2O$, 0.2 $(NH4)_2SO_4$, 0.02 $MgSO_4.7H_2O$, 0.001 $FeSO_4$, 1 LDPE, 20 agar, the Petri-dishes where incubated at 25 °c for one to two weeks. According to Raper & Fennell (1973) and Sheifert *et al*. (2011), the isolated fungi were identified.

The percentage of fungal occurance was recorded according to the following equation:

$$\text{appearrance percentage of the genus or species} = \frac{\text{number of samples in which the genus or species appeared}}{\text{total number of samples}} * 100$$

Study the enzymatic activity of the isolated fungi

Manganese peroxidase enzyme

The fungal isolate was inoculated onto Czapek-Dox agar medium (Hi media India) containing 0.0025% phenol red dye and incubated at 25°c for one week. A positive reaction is indicated by the transformation of the red color of the medium to the yellow color (Ali *et al*., 2012).

Lignase enzyme

The fungal isolate was inoculated onto PDA agar medium (Hi media India) containing % 0.5 methyl orange dye and incubated at 25°c for one week. A positive reaction is indicated by the transformation of the orange color of the medium to red color (Sharma *et al*., 2017).

Cellulase enzyme

The medium used for cellulose enzyme activity was contained g/l: 7.0 KH_2PO_4, 2.0 K_2HPO_4, 0.1 $MgSO_4.7H_2O$, 1.0 $(NH4)_2SO_4$, 0.6 yeast extract, 10-carboxy methylcellulose and 15 agar (Ireri *et al*., 2015). The fungal isolate was inoculated onto the previous medium for 7 days, and then the plates were flooded with 0.2% aqueous congo red solution and distained with 1M NaCl for 15 minutes. The appearance of yellow areas around the fungal colony in an otherwise red medium indicated cellulase activity.

Biodegradation of LDPE

Depending on the results of the enzymatic activity, the fungus *Aspergillus niger* was selected to study its ability to degrade LDPE. Pure fungal isolate was activated on PDA medium for one week before using, screening for a potential fungal isolate to degrade LDPE was carried out in

mineral salt broth medium (MSB) the composition of the culture media (g/l) was: 0.5 K_2HPO_4, 0.04 KH_2PO_4, 0.1 NaCl, 0.002 $CaC1_2.H_2O$, 0.2 $(NH4)_2SO_4$, 0.02 $MgSO_4.7H_2O$, 0.001 $FeSO_4$,10 glucose, the medium was autoclaved and supplemented by the pure fungal isolate and sterilized LDPE sheets. LDPE sheets were cut into small pieces 4 cm × 4 cm of similar weight, disinfected with 70% ethanol for 30 min, and transferred to sterile distilled water for 20 min before using three LDPE sheets (0.0266 g) were placed in each flask (Witt *et al*.,2001). Two types of control flasks were made, one containing MSB medium with LDPE sheets only and the other type containing the MSB medium with the fungal isolate without any LDPE sheets. All flasks sets were made in duplicate and incubated on a rotary shaker incubator at 25°c and 120 rpm for 90 days, the flasks were checked every 15 days for LDPE weight loss and the change in the fungal growth.

Measurement of LDPE weight loss

Measuring LDPE weight loss was made according to Gilian *et al*. (2004), LDPE sheets were weighed with a sensitive balance before biodegradation. After biodegradation, the LDPE sheets were washed with sterile distilled water for 20 min and then disinfected with 70% ethanol and washed again with sterile distilled water and left for 24h until it is dried and weighed again.

The following equation was used to measure the LDPE weight loss :

$$\text{LDPE weight loss}(\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} * 100$$

Statistical analysis

Minitab version 16 software was used to analyze the results using one-way analysis of variance. Relative least significant difference values were calculated to determine significant differences between the fungal processes. A completely randomized design was used.

Results and discussion

Isolates

Thirteen fungal species were isolated from eight soil samples (Table1). 69.23% of the isolated fungi belonged to the anamorphic fungi with nine species, followed by the zygomycota with 23.07 % with three species, and finally the ascomycetes fungi with only one species, 7.69%. The appearance of the anamorphic fungi in high percentage may be due to the ability of these fungi to produce a large number of reproductive units, and the secretion of different enzymes also, they possess a great ability to tolerate the stress in the environment, all of these features and others made them one of the large groups of fungi in the environment (Raaman *et al*., 2012).

Table 1: The isolated fungi with their percentage of occurrence

No.	Fungal species	% of occurrence
1	*Absidia* sp.	12.5
2	*Alternaria alternata*	25
3	*Aspergillus flavus*	87.5
4	*A. fumigatus*	37.5
5	*A. niger*	100
6	*A. terreus*	37.5
7	*A. versicolor*	25
8	*Chaetomium bostrychodes*	37.5
9	*Mucor* sp.	25
10	*Penicillium* sp.	50
11	*Rhizopou*s sp.	62.5
12	*stachybotry*s sp.	50
13	*Trichoderma* sp.	12.5

Enzymatic activity

The enzymatic activity of the isolated fungi where tested, the study of their ability to produce (manganese peroxidase, lignase and cellulase enzymes) rivaled clear variation between them in their ability to secrete these enzymes. It was observed that all fungi were able to show a variable enzymatic capability but in varying degrees and a varying number of enzymes that each fungus could secrete, which may be due to the inherent enzymatic activity of each fungus. It is well known that each microorganism possesses an enzymatic capacity that differentiated it from the other microorganisms (Sunitha *et al*., 2013; Patil *et al*., 2015). *Aspergillus flavus A. fumigatus, A. niger, A. terreus Penicillium* sp. and *stachybotrys* sp. have showed the ability to secrete all the studied enzymes, while the ability of the rest of the fungi ranged in their ability to secrete one to two enzymes.

The results have shown that most of the fungi in current study were able to produce the manganese peroxidase enzyme in which 11 fungal species were able to produce this enzyme with different capabilities of secretion rates from low as in *Alternaria alternata* to high secretion as in *Aspergillus niger* as showed in (Table 2, Fig.1). This enzyme is considered one of the most commonly used enzyme in converting toxic environmental pollutants into less toxic ones such as the degradation of tough plastic compounds. The fungi that can secrete this enzyme are distinguished by their ability to decompose complex pollutants and convert them into substances used as a source of energy and carbon. The ability of the fungus to secrete this enzyme becomes clear by changing the color of the medium from red to yellow because of the breakdown of the phenol aromatic ring (Varshney, 2013; Usha *et al*., 2014). Our result is consistent with Hui *et al*. (2017), who has found that 5 fungal isolates were able to secrete this enzyme.

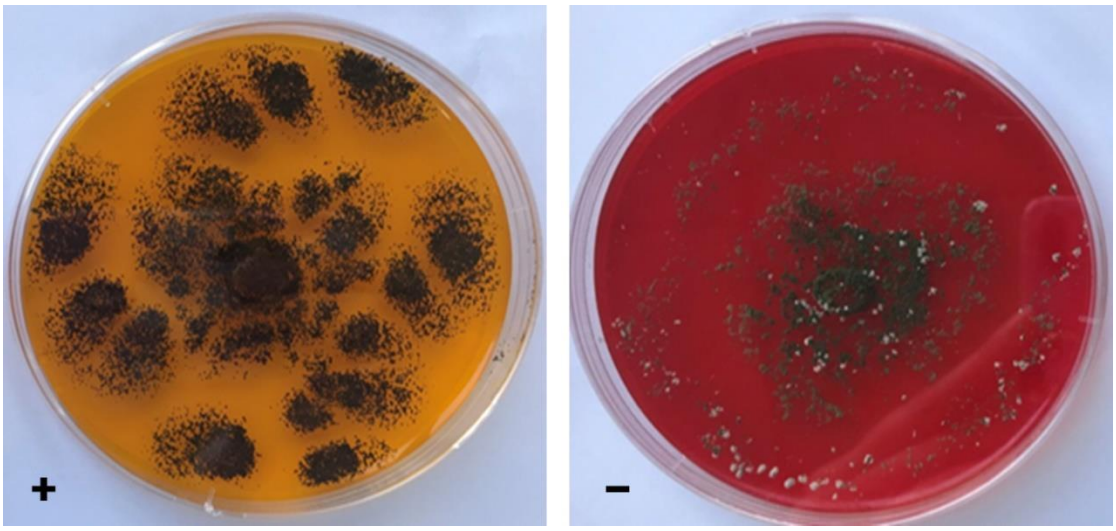

Fig. 1: Fungal enzymatic activity of manganese peroxidase enzyme (+) *Aspergillus niger*, (-) *Trichoderma* sp.

Table 2: Fungal enzymatic activity for manganese peroxidase enzyme

No.	Fungal species	Manganese peroxidase enzyme activity
1	*Aspergillus flavus*	+++
2	*A. fumigatus*	+++
3	*A. niger*	+++
4	*Penicillium* sp.	+++
5	*A. terreus*	++
6	*A. versicolor*	++
7	*Rhizopou*s sp.	++
8	*stachybotry*s sp.	++
9	*Absidia* sp.	+
10	*Alternaria alternata*	+
11	*Chaetomium bostrychodes*	+
12	*Mucor* sp.	-
13	*Trichoderma* sp.	-

-no secretion, **+:** medium secretion , ++ good secretion ,**+++:** high secretion.

Lignin is an essential component in the formation of vascular plant cells, and fungi play an important role in the degradation of this compound in the environment. The secretion of this enzyme from the fungi in this study indicates that these fungi play an important role in the degradation of these materials and possess great ability to explore it as a source of carbon and energy. In this study, 9 fungal species were able to produce lignase enzyme (Table 3, fig. 2). The secretion of this enzyme from the fungi indicates that these fungi play an important role in the degradation of the complicated material and can analyze many pollutants in the environment (Floudas *et al*., 2012). This is consistent with Bi *et al*. (2012), who has found that 72 fungal isolates were able to grow on the lignase- containing medium.

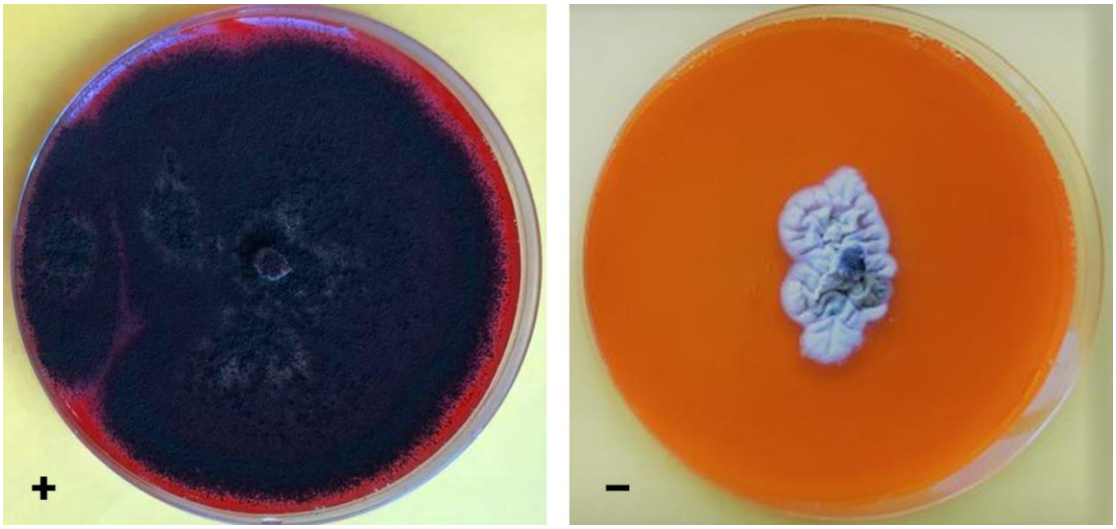

Fig. 2: Fungal enzymatic activity of lignase enzyme (+) *Aspergillus niger,* (-) *Chaetomium bostrychodes*

Table 3: Fungal enzymatic activity for lignase enzyme

No.	Fungal species	Lignase enzyme activity
1	*A. niger*	+++
2	*stachybotrys* sp.	+++
3	*Penicillium* sp.	++
4	*Rhizopous* sp.	++
5	*Aspergillus flavus*	+
6	*A. fumigatus*	+
7	*A. terreus*	+
8	*Chaetomium bostrychodes*	+
9	*Trichoderma* sp.	+
10	*Absidia* sp.	-
11	*Alternaria alternata*	-
12	*A. versicolor*	-
13	*Mucor* sp.	-

- no secretion, **+:** medium secretion , ++ good secretion ,**+++:** high secretion**.**

The cellulase enzyme comes in the third place with eight fungal species, which produced it from low as in *Absidia* sp. to high as in *A. niger*, as shown in (Table 4, Fig. 3). Most fungi that decompose plant parts can secrete cellulase enzymes. This enzyme gives a clear picture through the formation of a transparent halo around the colony due to the transformation of complex carbohydrates into simple sugars. It plays an important role in the activity of fungi, and the advantage of fungi that can

secrete this enzyme that they can convert toxic materials into less toxic materials to get rid of environmental pollution.

(Horn *et al*., 2012). This is consistent with Khalid *et al*. (2006), who has found that 42 fungal that is isolated could grow on the cellulase-containing medium.

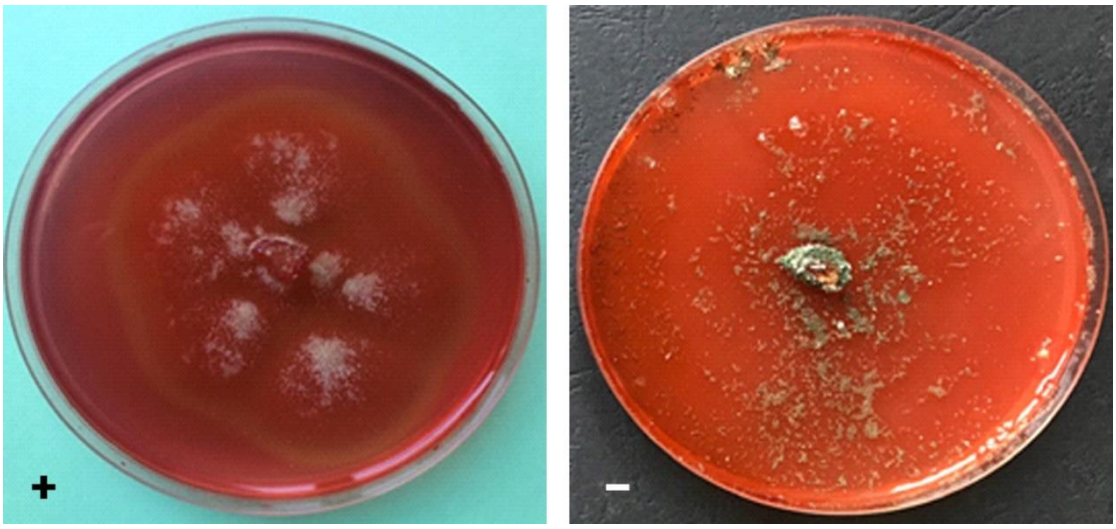

Fig. 3: Fungal enzymatic activity of cellulase enzyme (+) *Aspergillus terreus,* (-) *Trichoderma* sp.

Table 4 :Fungal enzymatic activity for cellulase enzyme

No.	Fungal species	cellulase enzyme activity
1	*A. niger*	+++
2	*A .terrus*	+++
3	*Penicillium* sp.	++
4	*stachybotrys* sp.	++
5	*Absidia* sp.	+
6	*Aspergillus flavus*	+
7	*A. fumigatus*	+
8	*Mucor* sp.	+
9	*Alternaria alternata*	-
10	*A .versicolar*	-
11	*Chaetomium bostrychodes*	-
12	*Rhizopous* sp.	-
13	*Trichoderma* sp.	-

-no secretion , + medium secretion (3-5) mm. **,** ++ good secretion (5-8) mm., +++ high secretion (8-11) mm.

Biodegradation of LDPE:

The fungus *Aspergillus niger* was chosen to study its ability to biodegrade LDPE; this fungus has showed good enzymatic activity for all tested enzymes and known for its ability to degrade many compounds in the environment (Immanuel *et al*., 2014).

The results showed that the percentage of loss for LDPE sheets were increased with time from 1.128% after 15 days of incubation to 26.128% after 90 days of incubation, significant differences for the percentage of loss was found between the incubation periods ($p<0.01$) (Fig. 4). The results showed that the rate of loss increased with time as a result of the adaption of the fungus to LDPE compound and start to use it as a source of energy and growth, especially after consuming all the glucose concentration in the medium, which stimulated the growth of the fungus and increased its enzymatic activity (Mohan and Suresh,2015; Parrales *et al*.,2018).

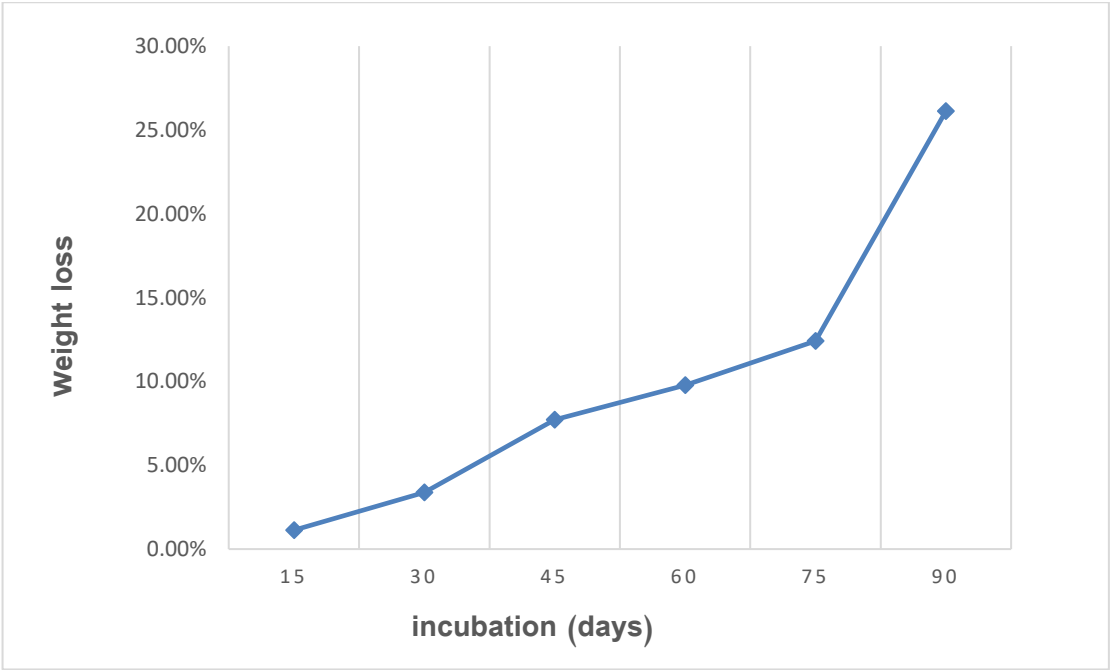

Fig. 4: Weight loss Percentage for the LDPE by *Aspergillus niger*

The dry weight of the fungus was also increased with times from 0.2142 g after 15 days of incubation compared with the control 0.0238 g to 0.4178 g after 90 days of incubation, significant differences was found between the dry weight of the fungus at 15 days and the end of the experiment ($p< 0.01$) (Fig. 5 & Fig. 6). Increasing fungal biomass with time results in an increase in the secretion of enzymes that degrade the LDPE compound, which becomes clear when the weight loss increases. Its assumed that high enzyme secretin yielded good fungal growth and increased its ability to biodegrade organic compounds (Nandi and Joshi,2013; Wang *et al*., 2018).

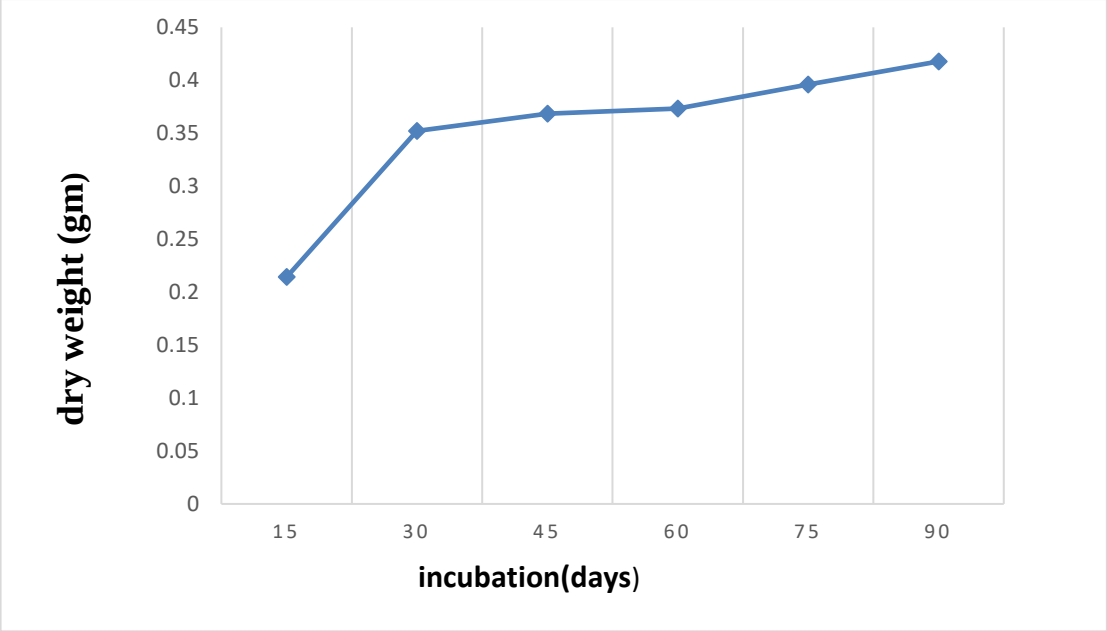

Fig. 5: Dry weight for the fungus *Aspergillus niger*

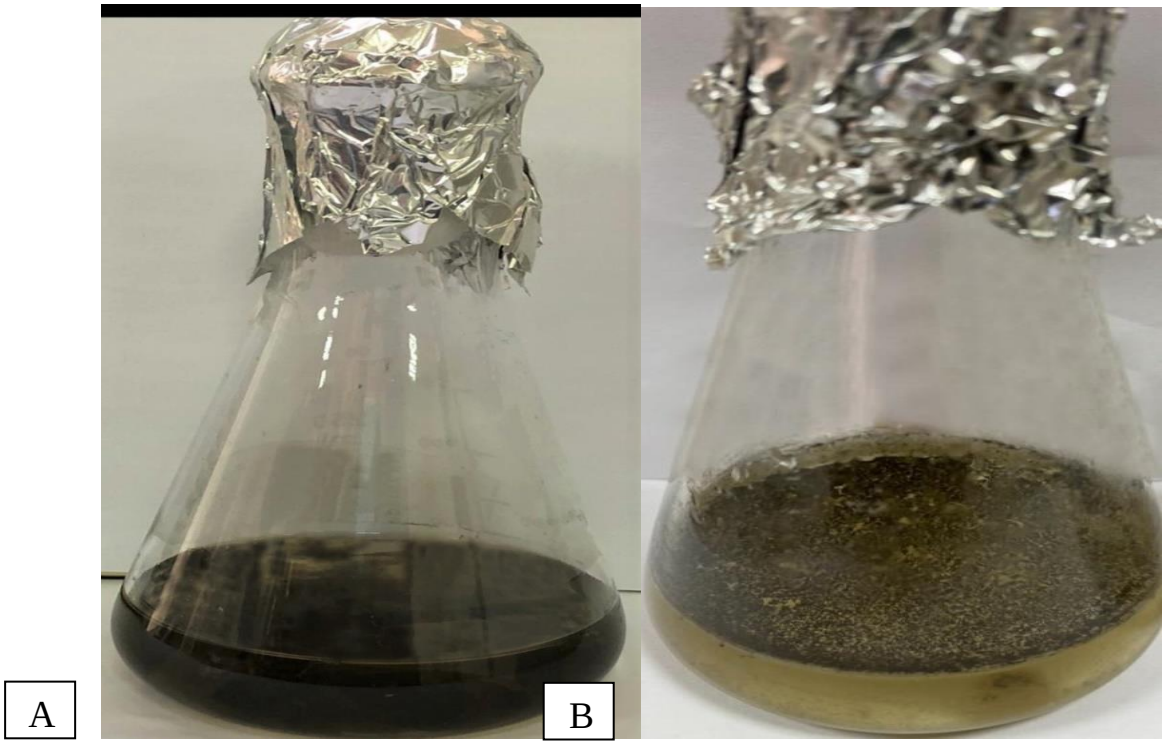

Fig. 6: Growth of *Aspergillus niger* in liquid medium. (a) growth with LDPE sheets. (b) growth in the control without LDPE sheets.

Conclusion

Some of the fungai isolated in this showed good enzymatic activity. This reflects that the fungi play an important role in the degradation of different organic materials in the environment; most of the isolated fungi appear a good ability to secrete manganese peroxidase enzyme, while cellulase enzyme came in the end with only eight species able to secrete this enzyme. The fungus *A. niger* has shown good ability to biodegrade LDPE compound, indicating the importance of using this fungus in the bioremediation process for this compound.

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تقييم النشاط الانزيمي لبعض الفطريات المعزولة من الترب الملوثة بالمخلفات البلاستيكية و قابليتها على التكسير الحيوي لمركب LDPE

مروة صبيح الطائي ومصطفى عبد الوهاب الدوسري

جامعة البصرة، كلية العلوم، قسم علم البيئة

المستخلص

خلال هذه الدراسة عزل ثلاثة عشر نوعاً فطرياً من ثمان عينات من الترب الملوثة بالمخلفات البلاستيكية التي تم جمعها من عدة اماكن في محافظة البصرة واختبرت فعاليتها الانزيمية لثلاث انواع من الانزيمات هي المنغنيز بيروكسيديز، اللكنيز والسيليلز. أظهرت الدراسة نشاط جيد للفطريات المعزولة، حيث تمكن 11 نوعا من الفطريات من انتاج انزيم المنغنيز بيروكسيديز، 9 انواع فطرية تمكنت من انتاج انزيم اللكنيز و ثمان انواع من الفطريات تمكنت من انتاج انزيم السيليليز، تم اختيار الفطر *Aspergillus niger* لدراسة قابليته على التكسير الحيوي لمركب LDPE في الاوساط السائلة ولمدة 90 يوما، اظهرت النتائج ان هذا الفطر كان قادرا على تحلل 26.128% من قطع LDPE بعد 90 يوماً من الحضن.