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# Biodegradation of plastic by some microorganisms as influenced by chemical factors

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ABSTRACT

This study examines microbial and enzymatic biodegradation of plastic, as well as some factors that influence their biodegradability. The results in the present work indicate that the plastic materials could be biodegraded by fungi, like Penicillium chrysogenum and Aspergillus niger for the use of the degradation products economically and the purified enzyme production in different industrial applications. Among 26 fungal isolates obtained, two isolates were identified by molecular tools *Penicillium chrysogenum* and *Aspergillus niger*. Different factors affect the degradation rate like carbon source, nitrogen source, metal salt inhibitors, and type of plastic materials. Comparing different carbon sources used in the fermentation medium of *Penicillium chrysogenum* and *Aspergillus niger*, glucose was the most suitable carbon source for maximum enzyme production for both fungal strains. Maximum enzyme activities were achieved after the addition of peptone at a concentration of 0.4 % and yeast extract at 0.6 % for two fungi, respectively, as an external supplement of nitrogen sources. Polylactic acid and Polycaprolactone were the most favorable type of plastic for the two fungi to degrade them. Zinc chloride slightly increased enzyme productivity. So, our Search for new fungal isolates capable of degrading plastic materials in soil.

## 1. Introduction

Plastics are polymers (solid materials) that become mobile when heated and can be cast into molds. They are nonmetallic moldable composites that can be molded into any shape and size needed [1]. Plastics are widely employed in a wide range of applications, including packaging, disposable diaper backing, agricultural films, and fishing nets [2]. Plastics and their applications have infiltrated every sector of the economy. Agriculture, telecommunications, building and construction, consumer goods, packaging, health, and medical are all high-growth industries that ensure current plastics need[3]. Natural plastics, these are renewable source products that biodegrade completely in their native state and their components from plants, animals, and algae. Natural polymers can be decomposed naturally[4]. Biodegradable plastics, a form of biopolymer, are produced by several bacteria and fungi. Polyhydroxyalkanoates (PHAs) are a good alternative to petrochemical plastics among the many biodegradable polymers since they are biodegradable, eco-friendly, and biocompatible [5]. Non-petroleum-based biological polyesters are projected to be among the most important next-generation polymers in the future due to limited natural resources [6]. PHAs have properties that are similar to polyethylene (PE) and polypropylene (PP) [7, 8]. When carbon is in excess of other nutrients such as nitrogen, sulfur, phosphorus, and oxygen, many microorganisms accumulate PHA as intracellular energy and carbon inclusion storage [9, 10].

PHAs can be classified into different types based on the number of repeating units in the polymers. Polymer-containing monomers of C3 to C5 hydroxyl fatty acids, such as polyhydroxybutyrate (PHB) and hydroxyvalerate, are known as short-chain-length PHA (scl-PHA) (HV). Similarly, medium-chain-length polymers are those made up of C6 to C16 hydroxyl fatty acids or aliphatic carbon sources (mcl-PHA) [11]. Heavy metals and antibiotics damage the environment naturally or through human activity and anthropogenic sources [12]. Heavy metal contamination from the environment in the fermentor has an impact on the production of PHA on an industrial scale[13]. Different scientists prefer to control contamination by using strains that are resistant to certain antibiotics [14]. PHAs are biodegradable polyesters produced by bacteria in response to environmental stress [15]. In a contaminated environment, a large number of bacteria can accumulate PHA as energy reserves. Polythene is the most commonly encountered non-

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biodegradable solid waste, and it has lately been discovered as a severe hazard to marine life [16]. Polythene may induce intestinal blockage in fish, birds, and marine mammals [17]. Polythene degradation is becoming a significant issue as the material becomes more commonly used [18]. In this paper, an attempt is made to isolate microorganisms that potentially degrade plastic materials from soil sediment [19]. Low-density polyethylene is a major contributor to environmental contamination [20]. Polyethylene is a long-chain ethylene monomer polymer [21]. The usefulness of polyethylene is increasing at a 12% annual pace, and around 140 million tonnes of synthetic polymers are manufactured globally each year [12].

With so much polyethylene in the environment, its disposal presents serious environmental concerns. They deteriorate over thousands of years. In the presence of living creatures, biodegradable polymers deteriorate. Decomposition of biodegradable polymers occurs in a variety of conditions [22]. Depolymerisation is produced by some physical and biological factors [23]. Mechanical damage to the polymer is caused by physical forces such as temperature, moisture, and pressure. Microbial biodegradation is widely acknowledged and still used because of its increasing efficiency.

Several microorganisms have recently been discovered to generate degrading enzymes, and some enzymatic activities cause microbial degradation of plastics, resulting in polymer chain cleavage into oligomers and monomers [24]. Aerobic metabolism produces carbon dioxide and water [25]. Anaerobic metabolism produces carbon dioxide, water, and methane, which are referred to as end products, respectively [26]. Degradation causes polymers to break down into monomers, making it easier for microbial cells to accumulate for further degradation [27].

The primary goal is to isolate and characterize soil microorganisms that use plastic materials as their only source of carbon and nitrogen.

## 2. Materials and Methods

### 2.1. Microorganisms Used

In this study, two fungal strains namely, *Penicillium chrysogenum* and *Aspergillus niger*, were used. These strains were obtained from the Mycological Laboratory, Botany Department, Faculty of Science, Fayuom University. The stoke cultures of the tested strains were maintained on Czapek's dox agar slants at 4 °C and sub-cultured monthly.

### 2.2. Quantitative Lignin Peroxidase Assay

Methylene Blue Assay: The assay mixture contained in 3.0 ml: 2.2 ml of the diluted supernatant, 0.1 ml of 1.0 mM methylene blue, and 0.6 ml of 0.5 M sodium tartrate buffer (pH 4.0). The reaction was started by the addition of 0.1 ml of 2.7 mM $H_2O_2$. The conversion of the dye to Azure C was monitored by the measurement of the decrease in absorbance at 664 nm. The data were reported as changes in absorbance per minute, and the activities as U/L.

### 2.3. Effect of different Carbon sources on enzyme production

The experiment was designed to find out the best carbon source that produces the highest amount of enzyme by *P. chrysogenum* and *A. niger*. Different carbon sources namely, glucose, sucrose, maltose, lactose, fructose, and starch tested as the sole carbon source in the fermentation medium at a concentration of 2 % (W/V). Two-hundred and fifty ml Erlenmeyer flasks each of which contains 100 ml of mineral salt medium were sterilized for 20 min. at 121°C after the addition of 1 % plastic materials to it. Separately sterilized external carbon sources were aseptically added to the fermentation medium at a final concentration of 2% (W/V), pH of the fermentation medium was adjusted to 5 for P. chrysogenum and pH 6 for A. niger. Flasks containing mineral salt medium without the added carbon source were used as control. Each flask was inoculated with the test fungal species *P. chrysogenum* and *A.niger* separately, which were previously grown on Czapek's glucose agar at 25°C for 7 days. Flasks were incubated for 21 days (the best incubation period) at 40°C for *P. chrysogenum* and 35°C for *A.niger*. The best-added carbon source (glucose) for both fungal species then, was examined at different concentrations, 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 % on the mineral salt medium to examine the effect of glucose concentration on enzyme activity. Triplicate flasks inoculated with fungal discs. The enzyme activity was measured using the culture filtrate after the incubation period.

### 2.4. Effect of different nitrogen sources on enzyme production

250 ml Erlenmeyer flasks containing 100 ml of mineral salt medium were sterilized at 121°C for 20 minutes before being filled with 1% plastic material. The external nitrogen sources (peptone, yeast extract, potassium nitrate, sodium nitrate, ammonium chloride, and ammonium phosphate) were added to the fermentation medium at a final concentration of 0.2 % (W/V). pH of the fermentation medium was adjusted at 5.0 for P. chrysogenum and 6.0 for *A. niger* before autoclaving. Medium-free from added nitrogen source was used as control. After autoclaving, each flask was inoculated with discs of the test fungal species, *P. chrysogenum* and *A. niger* separately, that were previously grown on Czapek's glucose agar at 25°C for 7 days. Flasks were incubated for 21 days (the best incubation period) at 40°C for P.chrysogenum and 35°C for *A. niger*.

In the second part of the experiment, the best-added nitrogen source (yeast extract for *A. niger* and peptone for *P. chrysogenum*) for enzyme production obtained from the first part of the experiment, was added to the mineral salt medium at the following concentrations, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 g%. Triplicate flasks were inoculated with fungal discs. The liquid medium with 20 g/l of the chosen carbon source as the best carbon concentration from the previous experiment was employed. After the incubation period, at 40°C for *P.chrysogenum* and 35 °C for *A. niger*, The enzyme activity was measured using the culture filtrate.

### 2.5. Effect of metal salt inhibitors on enzyme activity

One hundred ml mineral salt medium in 250 ml Erlenmeyer flasks was adjusted at pH 5 and pH 6 for *P.chrysogenum* and *A.niger*, respectively, with the best carbon and nitrogen sources added at optimum concentration (the control medium). 1 % plastic material together with metal inhibitors was added to the medium after autoclaving, the metals being used were $ZnCl_2$, $MnCl_2.4H_2O$, $CuCl_2.2H_2O$, $CoCl_2.6H_2O$, $CaCl_2.2H_2O$ and $CdCl_2.H_2O$, at a concentration of 10 µM. Whereas ethylene diamine tetra acetic acid (EDTA) was added at a concentration of 0.5 mM. Fungal discs were sown in triplicate flasks. Flasks were incubated at 35°C for *A. niger* and 40°C for *P.chrysogenum* for 21 days. The culture medium filtrate was used for the estimation of

enzyme activity.

2.6. Effect of different types of plastic materials on enzyme production

Plastic wastes (polyethylene, polyvinylchloride polyurethane, polycaprolactone, and polylactic acid) were fragmented into 1 cm long pieces and added to mineral salt media as the sole carbon and nitrogen source to study the biodegradation of different types of plastic materials. Plastic materials were added separately to 100 ml of the mineral salt medium in 250 Erlenmeyer flasks at the rate of 1 % after autoclaving at 121°C for 20 min, flasks were inoculated with each of the tested fungal isolates and incubated for 21 days at 40°C for *P. chrysogenum* and 35°C for *A. niger*. All other optimum conditions were used. The plastic degradation assay was determined after the incubation periods..

**3. Results and discussion**

3.1. Effect of different carbon sources on enzyme production

*Penicillium chrysogenum* and *Aspergillus niger* were grown for three weeks on the basal salt medium containing 100 mg plastic materials. The following carbon sources were added to the incubation medium as a supplement carbon source, glucose, sucrose, fructose, lactose maltose, and starch to test their effect on enzyme activity. The initial pH of the incubation medium was adjusted at pH 5 and pH 6 for *Penicillium chrysogenum* and *Aspergillus niger*, respectively. Flasks were incubated at incubation temperatures of 40°C and 35°C for two fungal species, respectively. The enzyme activity was measured at the end of the incubation period. The presence of these carbon sources in the growth medium exerted a stimulative effect on enzyme production whereas starch did not exhibit a noticeable effect. Glucose was the most powerful carbon source for inducing the enzyme activity, this was associated with the highest increase in biomass accumulation compared to the control free from glucose. The preference for glucose may be due to its ready availability for glycolysis by the fungi. The data presented in Table (1) showed that glucose was the best carbon source for enhancing fungal growth and enzyme production compared with the control. The present results are in agreement with [28]**.** The *P. chrysogenum* activity was 23.7 U/ml when glucose was added to the fermentation medium, while it was 25.3 U/ml for *A.niger*. On the other hand, starch gave the lowest fungal growth of *Penicillium* and the least enzyme activity (11.0 U/ml). Sucrose also gave good fungal growth for *A. niger* and *P.chrysogenum*. Starch on the other hand had the lowest effect in enhancing the *A. niger* growth and enzyme activity compared with the control [29, 30]. Some added carbon sources increased in final pH compared with the initial pH of the growth medium [31]. Glucose was demonstrated to be the optimum carbon source for maximal enzyme activity by *P. chrysogenum* and *A. niger* consequently it was used in different concentrations to examine the effect of glucose concentration on enzyme activity.

**Table 1.** Effect of supplementation of the mineral salt medium with external carbon source on final pH of the cultivation medium, fungal dry weight, and enzyme activity of *Penicillium chrysogenum* and *Aspergillus niger,* at suitable pHs and temperatures

| External carbon source | Final pH | | Dry Weight (mg/100ml) | | Enzyme activity (U/ml) | |
|---|---|---|---|---|---|---|
| | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* |
| No Carbon source | 7.3±0.17 | 8.1±0.06 | 133.6 ±0.06 | 145.9±0.06 | 7.9±0.06 | 9.0±0.06 |
| Glucose | 8.2±0.12 | 8.6±0.12 | 245.8±0.12 | 250.0±1.15 | 23.7±0.12 | 25.3±0.17 |
| Sucrose | 6.6±0.12 | 6.70.06 | 214.2±0.12 | 210.0±0.58 | 11.5±0.29 | 15.3±0.17 |
| Fructose | 6.8±0.12 | 7.5±0.29 | 211.9±0.12 | 186.7±0.07 | 12.0±0.12 | 14.0±0.58 |
| Lactose | 6.8±0.06 | 6.5±0.29 | 200.0±0.58 | 170.0±0.58 | 12.0±0.58 | 13.0±0.29 |
| Maltose | 6.6±0.06 | 6.5±0.29 | 157.0±1.15 | 158.0±0.58 | 13.2±0.12 | 17.0±0.29 |
| Starch | 6.5±0.06 | 6.4±0.23 | 120.6±0.12 | 209.7±0.12 | 11.0±0.12 | 9.8±0.06 |
| LSD ( at 0.01) | 0.522 | | 1.720 | | 0.706 | |
| LSD ( at 0.05) | 0.386 | | 1.273 | | 0.523 | |

LSD; least significant difference.

3.2. Effect of different concentrations of glucose on enzyme production.

From the above-mentioned results in Table (1) glucose as a sole carbon source gave the highest enzyme yield. Therefore, it was necessary to establish the effect of different concentrations ( 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0%) on the production of enzymes by tested fungal strains. Data presented in Table (2) indicated that the highest enzymatic activity was produced in a medium containing 0.5% and 1.0% glucose for *P. chysogenum* and *A. niger*, respectively at these concentrations the enzymatic activity was 27.0 U/ml for *P. chysogenum* and 33.7 U/ml for *A. niger* below or above these concentrations a marked drop in enzyme production was observed. Glucose supplementation to the culture medium of degraded fungi accelerated growth and increased the maximum dry mass. These results are in agreement with [32]**.**

3.3. Effect of different nitrogen sources on enzyme production by tested fungal strains

*Penicillium chrysogenum* and *Aspergillus niger* were cultivated for three weeks on the basal salt medium containing 100 mg plastic materials with a suitable concentration of glucose. As an external supplement nitrogen sources at 0.2% concentration, yeast extract, peptone, Ammonium chloride, potassium nitrate, ammonium phosphate, and sodium nitrate were used to test their effect on enzyme activity. The pH of the incubation medium was initially adjusted at 5 and 6 for *P. chrysogenum* and *A. niger*, respectively. The flasks were incubated at optimum temperatures of 40°C and 35°C for *P. chrysogenum* and *A. niger*, respectively. Enzyme activity was measured at the end of the incubation time. Data in Table (3) showed that peptone was the best nitrogen source added to the fermentation medium for inducing enzyme activity of *P. chrysogenum* where the enzyme activity was (26.0 U/ml), while yeast extract was the best nitrogen source for the enzyme activity of *A. niger* where the enzyme activity which was (29.0 U/ml). Sodium nitrate also improved enzyme activity compared with fermentation medium with no external nitrogen supply (control) with values of 19.0 and 21.4 U/ml for *P. chrysogenum* and *A. niger,* respectively [33 - 35]. Peptone was then employed in other studies at various concentrations to examine the effect of its

concentration on the enzyme activity of *P. chrysogenum* and yeast extract for *A. niger* Table(4). It is worthy to mention that all added nitrogen sources either organic or inorganic in the growth media were stimulative for fungal growth.

**Table 2.** Effect of different concentrations of glucose on the final pH, fungal dry weight, and enzyme activity of *Penicillium chrysogenum* and *Aspergillus niger*, at suitable pHs and temperatures

| Glucose conc. % | Final pH | | Dry Weight (mg/100 ml) | | Enzyme activity (U/ml) | |
|---|---|---|---|---|---|---|
| | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* |
| 0.25 | 7.3±0.17 | 7.9±0.12 | 210.0±1.15 | 209.7±0.12 | 12.3±0.17 | 13.0±0.17 |
| 0.5 | 7.5±29 | 7.7±0.12 | 183.0±1.73 | 245.7±0.12 | 27.0±0.58 | 25.5±0.29 |
| 0.75 | 7.2±0.12 | 7.5±0.29 | 215.9±0.12 | 222.0±1.15 | 23.0±0.58 | 29.7±0.12 |
| 1 | 7.2±0.12 | 7.2±0.06 | 198.5±0.29 | 235.5±0.29 | 24.0±0.58 | 33.7±0.12 |
| 1.5 | 7.1±0.06 | 7.0±0.12 | 188.5±0.29 | 195.8±0.12 | 17.0±0.58 | 21.5±0.29 |
| 2 | 6.2±0.12 | 6.4±0.23 | 200.5±0.29 | 156.7±0.12 | 13.7±0.06 | 18.8±0.06 |
| LSD ( at 0.01) | 0.317 | | 2.223 | | 1.300 | |
| LSD ( at 0.05) | 0.234 | | 1.636 | | 0.957 | |

LSD; least significant difference.

**Table 3**. Effect of the addition of different nitrogen sources to the incubation medium on the final pH of the cultivation medium, fungal dry weight, and enzyme activity of *Penicillium chrysogenum* and *Aspergillus niger*, at suitable pHs and temperatures.

| Nitrogen source | Final pH | | Dry Weight (mg/100 ml) | | Enzyme activity (U/ml) | |
|---|---|---|---|---|---|---|
| | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* |
| No nitrogen source added | 8.0±0.23 | 7.8±0.23 | 198.0 ±1.15 | 210.0±1.15 | 18.0±0.58 | 16.0±0.58 |
| Yeast extract | 8.3±0.17 | 8.6±0.17 | 155.6±1.15 | 188.7±0.40 | 25.0±1.15 | 29.0±1.53 |
| Peptone | 8.5±0.12 | 8.3±0.12 | 199.0±0.58 | 230.0±1.15 | 26.0±0.58 | 27.0±0.58 |
| $NH_4Cl$ | 7.3±0.17 | 7.3±0.12 | 188.4±0.23 | 189.4±0.23 | 12.0±0.58 | 14.0±0.58 |
| $KNO_3$ | 7.5±0.29 | 8.1±0.12 | 214.0±0.58 | 198.3±0.17 | 17.2±0.12 | 20.0±1.15 |
| $(NH_4)_3PO_4$ | 8.1±0.06 | 7.3±0.17 | 185.0±1.15 | 177.6±0.35 | 15.0±1.33 | 15.0±0.58 |
| $NaNO_3$ | 8.1±0.06 | 8.0±0.06 | 210.0±1.15 | 211.8±0.46 | 19.0±0.58 | 21.4±0.23 |
| LSD ( at 0.01) | 0.336 | | 1.881 | | N.S. | |
| LSD ( at 0.05) | 0.249 | | 1.392 | | 2.130 | |

LSD; least significant difference, N.S.: not significant.

3.4. Effect of different concentrations of the best nitrogen sources on enzyme production.

Data from Table (4) showed that enzyme activity was stimulated by the increase in peptone concentrations up to 0.4 % after that it gradually decreased as the concentration of peptone increased. For *Aspergillus niger*, yeast extract was the best nitrogen source. Ammonium chloride and ammonium phosphate have the least effect on enzyme activity. Values of pH were moved to alkalinity by employing yeast extract, peptone, potassium nitrate, and sodium nitrate. When yeast extract was added subsequently to the fermentation medium in different concentrations enzyme activity was stimulated by the increase in yeast extract concentrations up to 0.6 % Table (4), after such concentrations the enzyme activity declined [36].

**Table 4**. Effect of the addition of nitrogen source concentrations to the incubation medium on the final pH of the cultivation medium, fungal dry weight, and enzyme activity of Penicillium chrysogenum and Aspergillus niger, at suitable pHs and temperatures.

| Nitrogen source concentration % | Final pH | | Dry Weight (mg/100 ml) | | Keratinase activity (U/ml) | |
|---|---|---|---|---|---|---|
| | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* |
| 0.2 | 8.1±0.06 | 8.3±0.17 | 204.6±0.35 | 220.0±1.15 | 23.0±0.58 | 24.0±1.15 |
| 0.4 | 8.2±0.12 | 8.4±0.23 | 211.1±0.06 | 221.2±0.12 | 24.0±0.58 | 25.4±0.23 |
| 0.6 | 8.3±0.17 | 8.5±0.29 | 211.3±0.17 | 234.6±0.35 | 21.6±0.35 | 27.0±1.15 |
| 0.8 | 8.4±0.23 | 8.5±0.29 | 212.0±1.15 | 240.8±0.46 | 22.2±0.12 | 21.1±0.06 |
| 1 | 8.5±0.12 | 8.6±0.06 | 212.1±0.06 | 240.9±0.52 | 19.6±0.07 | 19.5±0.29 |
| 1.2 | 8.5±0.12 | 8.7±0.12 | 212.8±0.46 | 245.2±0.12 | 20.9±0.52 | 18.0±0.58 |
| LSD ( at 0.01) | N.S. | | 1.743 | | 1.494 | |
| LSD ( at 0.05) | N.S. | | 1.282 | | 1.099 | |

LSD; least significant difference, N.S.: not significant.

3.5. Effect of metal salts and EDTA on enzyme production by tested fungal strains

Data in Table (5) show the influence of some metal salts in concentrations (10μM) and EDTA (0.5mM) on enzyme activity after 21 days of incubation on the basal medium at pH 5 with 0.5 % glucose and 0.4 % peptone at 40°C for *Penicillium chrysogenum*. The results revealed that all of the metals added to the medium inhibited enzyme activity to different degrees [37]. The maximum inhibition was produced by using $CaCl_2$ and $MgCl_2$, which reduced enzyme activity to approximately half of its original value. The enzyme activity in the control treatment was 24.0 U/ml, whereas that of $CaCl_2$ and $MgCl_2$ was 18.0 and 14.5 U/ml, respectively. Zinc chloride and cobalt chloride have very little effect on the enzyme activity where the enzyme activity was 23.0 and 22.9 U/ml, respectively. $MgCl_2$ and $ZnCl_2$ also enhance the fungal dry weight. Also, data in Table (5) show the effect of some metal salts and EDTA on enzyme production of *Aspergillus niger* after 21 days of incubation at 35 ºC and the basal medium pH 6, with 1.0 % glucose and 0.6 % yeast extract was used, some of these compounds exhibited inhibition to enzyme production as calcium chloride, manganese chloride, copper chloride, cadmium chloride, and ETDA. The rest of the metal salts and EDTA were enzyme repressors. Some salts, such as cobalt chloride and zinc chloride, have

no effect on enzyme production. Zinc chloride noticed increases in enzyme production (28.6 U/ml) with the findings obtained by [38 - 40].

**Table 5**. Effect of some heavy metal salts (10μM) and EDTA (0.5mM) on the final pH of the cultivation medium, fungal dry weight, and enzyme activity of *Penicillium chrysogenum* and *Aspergillus niger*, at suitable pHs and temperatures.

| Metal | Final pH | | Dry Weight (mg/100 ml) | | Enzyme activity (U/ml) | |
|---|---|---|---|---|---|---|
| | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* |
| Control | 8.1±0.06 | 8.6±0.12 | 225.8±0.46 | 210.7±0.40 | 24.0±0.58 | 28.0±0.58 |
| $MgCl_2$ | 8.0±0.12 | 7.0±0.29 | 243.4±0.23 | 222.5±0.29 | 14.5±0.29 | 19.0±0.58 |
| $CuCl_2$ | 7.2±0.12 | 7.4±0.23 | 217.7±0.40 | 211.6±0.35 | 21.0±1.15 | 17.0±0.58 |
| $CoCl_2$ | 6.7±0.09 | 6.8±0.12 | 190.8±0.52 | 224.6±0.35 | 22.9±0.52 | 16.0±0.33 |
| $CaCl_2$ | 6.5±0.29 | 7.8±0.12 | 210.5±0.29 | 213.5±0.29 | 18.0±0.58 | 15.0±1.15 |
| $CdCl_2$ | 6.9±0.06 | 8.1±0.06 | 227.7±0.40 | 220.0±0.58 | 21.0±0.58 | 24.0±1.15 |
| $ZnCl_2$ | 7.1±0.06 | 7.5±0.29 | 232.0±1.15 | 280.4±0.23 | 23.0±1.73 | 28.6±0.35 |
| EDTA | 7.0±0.29 | 7.6±0.17 | 205.7±0.40 | 201.5±0.29 | 23.0±1.15 | 28.0±0.58 |
| LSD ( at 0.01) | 0.405 | | 1.341 | | 2.501 | |
| LSD ( at 0.05) | 0.301 | | 0.996 | | 1.857 | |

LSD; least significant difference.

3.6. Effect of some types of plastic materials on enzyme production.

*Penicillium chrysogenum* and *Aspergillus niger* were grown for three weeks on a mineral salt medium. Different types of plastic wastes namely, (polyethylene, Polyvinylchloride, Polyurethane, Polycaprolactone, and Polylactic acid) were cut into 1.0 cm pieces and added to the fermentation media as the sole source of carbon and nitrogen. Optimum conditions were employed for each of the examined fungi. Enzyme activity was measured at the end of the experiment. The obtained results are represented in Table (6). The data showed that the tested fungi actively hydrolyzed different plastic wastes to a different extent [41]. The enzyme activity was greatly affected by the source of the plastic waste materials [42 - 44]. Polycaprolactone and Polylactic acid gave the highest enzyme activity for *P. chrysogenum* (14.6 U/ml) and (13.6 U/ml), due to the two polymers being natural plastics, while Polyvinylchloride gave a very low enzyme activity (6.7 U/ml) for P. chrysogenum. The highest enzyme activity for *A.niger* was obtained in the presence of Polylactic acid and Polycaprolactone as source material where the enzyme activity value was (17.9 u/ml and 15.6 u/ml), respectively [45, 46].

**Table 6.** Effect of different types of plastic on final pH of the cultivation medium, fungal dry weight, and enzyme activity of *Penicillium chrysogenum* and *Aspergillus niger*, at suitable pHs and temperatures

| Type of plastic | Final pH | | Dry Weight (mg/100 ml) | | Enzyme activity (U/ml) | |
|---|---|---|---|---|---|---|
| | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* | *Penicillium* | *Aspergillus* |
| Control | 6.0±0.58 | 6.5±0.29 | 70.0±2.89 | 75.0±2.89 | 5.5±0.29 | 8.6±0.12 |
| Polyethylene | 8.5±0.29 | 8.6±0.21 | 77.8 ±0.52 | 84.6±0.35 | 9.7±0.12 | 9.0±0.12 |
| Polyvinylchloride | 8.5±0.17 | 8.4±0.10 | 100.0±1.15 | 111.6±0.35 | 6.7±0.09 | 10.3±0.17 |
| Polyurethane, | 8.2±0.15 | 8.0±0.32 | 88.4±0.23 | 98.5±0.29 | 8.5±0.29 | 11.2±0.12 |
| Polycaprolactone | 8.6±0.54 | 8.5±0.28 | 120.3±0.17 | 145.5±0.29 | 14.6±0.17 | 15.6±0.23 |
| Polylactic acid | 8.1±0.19 | 8.5±0.26 | 125.8±0.46 | 142.9±0.52 | 13.6±0.60 | 17.9±0.23 |
| LSD ( at 0.01) | N.S | | 3.982 | | 0.727 | |
| LSD ( at 0.05) | N.S | | 2.929 | | 0.535 | |

LSD; least signifat difference, N.S.: not significant

## 4. Conclusion

The results in the present work indicate that the plastic materials could be biodegraded by fungi, like *Penicillium chrysogenum* and *Aspergillus niger* for the use of the degradation products economically and the purified enzyme production in different industrial applications.

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