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Full Length Research Paper

Biodegradation of low density polyethylene (LDPE) by fungi isolated from marine water– a SEM analysis

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Fungi, isolated from sea water, were subjected to growth in a medium containing low density polyethylene (LDPE) as the sole carbon source with and without yeast extract. Increasing fresh weight of the fungi in the medium supplemented with LDPE after regular time intervals gave the evidence that the fungi were utilizing LDPE as the carbon source. Further confirmation of LDPE utilization was carried out by the Sturm test where the degradation was attributed to the amount of carbon dioxide evolved during the growth period. The two fungi that showed good growth in medium supplemented with LDPE proved to degrade LDPE with higher efficiency in earlier reported results Scanning Electron Microscopy analysis of the fungal treated LDPE films provided a solid evidence of biodegradation. Fungi were identified as *Aspergillus* spp. LDPE degradation is a severe environmental crisis in the world and we have proved that microorganisms can be used for bioremediation in this line.

Key words: Biodegradation, low density polyethylene (LDPE), *Aspergillus*, marine fungi, Sturm test.

INTRODUCTION

The world plastic comes from the Greek word “plastikos” which means able to be molded into different shapes. The plastics we use today are made from inorganic and organic raw materials, such as carbon, silicon, hydrogen, nitrogen, oxygen and chloride. The basic materials used for making plastics are extracted from oil, coal and natural gas.

Polyethylene (PE) is a thermoplastic polymer consisting of long chains produced by combining the ingredient monomer ethylene. The ethylene actually converts to ethane as it takes its place in a polymer and straight sections of the polymers are the same structure as the simple chain hydrocarbons. The most important polyethylene grades are HDPE, LLDPE and LDPE. LDPE is defined by a density range of 0.910-0.940 g/cm^3. It is not reactive at room temperature, except by strong oxidizing agents. It can withstand temperatures at 80°C continuously and 95°C for a short time. It is translucent or opaque variations; it is quite flexible and tough but breakable. LDPE has more branching (on about 2% of the carbon atoms). Its intermolecular forces are weaker, its tensile strength is lower and its resilience is higher. Its molecules are less tightly packed and less crystalline; because of its side branches, its density is lower. LDPE contains chemical elements, carbon and hydrogen. It shows excellent resistance to dilute and concentrated acids, alcohols, bases and esters, good resistance to aldehydes, ketones and vegetable oils, limited resistance to aliphatic and aromatic hydrocarbons, minerals oils and oxidizing agents and poor resistance and not recommended for use with halogenated hydrocarbons.

Among synthetic plastics, the most problematic one in this regard is polyethylene. The resistance of polyethylene to biological attack is related to its hydrophobicity and water repellency polyethylene-considered to be inert can be biodegraded if the right

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microbial strain is isolated. The biodegradation of polyethylene is a slow process. Microorganisms such as bacteria, fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics (Gu et al., 2000a). The degradation of most synthetic plastics in nature is a very slow process that involves environmental factors followed by the action of wild microorganisms (Albertsson, 1980). Biodegradation of polyethylene has been studied extensively earlier (Albertsson, 1980; Breslin, 1993; Breslin and Swanson, 1993; Imam and Gould, 1990) but the results were based on polyethylene blended with starch. Lee et al. (1991) have reported the biodegradation of degradable plastic polyethylene by *Phanerochaete* and *Streptomyces* species. Fourst et al. (1997) have reported the biodegradation of LDPE/Cellulose blends by common fungi. El-Shafei et al. (1998) have reported the biodegradation of disposable polyethylene by fungi and *Streptomyces* species. A major obstacle to biodegradation of the polyethylene is the resistance of LDPE to biological attack because of its hydrophobicity, high molecular weight and its lack of functional groups recognized by microbial enzymatic systems (Hamid, 2000). Thermally treated LDPE was proved to be biodegradable by *Pencillium pinophilum* and *Aspergillus niger.* Kathiresan (2003) has reported isolating fungi from the mangrove soil which has the potential to degrade polyethylene materials. In most studies, fungi were considered for the degradation of LDPE due to their ability to form hydrophobic proteins that can attach to the polymer surface (Seneviratne et al., 2006; Kershaw and Talbot, 1998), their generation of degrading enzymes that are well matched to the insoluble LDPE (Shah et al., 2008), the faster growth of fungal biomass compared to bacteria (Kim and Rhee 2003) and the growth extension and penetration into other locations through the distribution of hyphae. Also fungi survive environments with low nutrient availability, low pH and low moisture well. Yamada-Onodera et al. (2001) isolated a strain of fungus *Penicillium simplicissimum* YK to bio-degrade polyethylene without additives. El-Shafei et al. (1988) investigated the ability of fungi and *Streptomyces* strains to attack degradable polyethylene consisting of disposed polyethylene bags containing 6% starch. Still a lot has to be done to isolate the right kind of microbial strain that could promote degradation of LDPE in a shorter period of time since all the previous reports showed activity only after a minimum period of 3-4 months. Several analytical methods have been used to test biodegradability which includes visual observation, changes in molar mass, weight loss measurement, CO_2 evolution, clear zone formation etc. Under aerobic conditions, microbes use oxygen to oxidize carbon and form carbon dioxide as one major metabolic end product. Consequently, the formation of carbon dioxide (Sturm test) is a good indicator for polymer degradation and is the most often used method to measure biodegradation in laboratory tests. This test has long been used to evaluate the degradability of diverse substances and chemicals in water (OECD guidelines) and has now been adapted to applications in non-water soluble polymeric materials like the trapping of the CO_2 gas in KOH solution and performing the gravimetric analysis with the help of Barium chloride solution as suggested by Muller et al. (1992).

MATERIALS AND METHODS

Sea water sample was collected from Kovalam coast-off the Bay of Bengal, 500 m away from shore at the depth of 5 m. Sample was inoculated in Malt Extract Soyapeptone Broth After observation of visible growth, the inoculum was transferred to Soyapeptone Medium having LPDE in the powdered form (0.50 g/100 ml).

Preparation of LDPE powder

LDPE sheets were cut into bits and immersed in xylene. It was boiled for 15 min as xylene dissolved the LDPE film and the residue was crushed while it was warmed by using band gloves. The LDPE powder so obtained was washed with ethanol to remove residual xylene and allowed to evaporate to remove ethanol. The powder was dried in hot air oven at 60^{o}C over night.

Fungal colonizing studies

The colonizing capacity of the fungi on LDPE film was studied in big sized Petri-plate. Briefly, Mineral Salt Medium (MSM) containing the following salts in 1 L distilled water: K_2HPO_4, 1 g; KH_2PO_4, 0.2 g; NaCl, 1 g; $CaCl_2.2H_2O$, 0.002 g; boric acid, 0.005 gm; $(NH_4)_2SO$, 1 g; $MgSO_4.7H_2O$, 0.5 g; $CuSO_4.5H_2O$, 0.001 g; $ZnSO_4.7H_2O$,0.001 g; $MnSO_4.H_2O$, 0.001 g and $FeSO_4.7H_2O$, 0.01 g was aseptically poured into Petriplates. LDPE sheets were cut into small pieces 2 cm × 2 cm of similar weight, disinfected with 70% ethanol for 30 min and transferred to sterile distilled water for 20 min. Five LDPE sheets of same weight were placed in Petriplates containing the Minimal Salt Medium without yeast extract which was inoculated with 5 similar sized colonies of fungi using the cork borer. The Petriplates were incubated at room temperature and results were observed after 1 week to 10 days.

Quantification of CO_2 – Modified Sturm test (Muller et al., 1992)

100 ml capacity autoclavable plastic containers were used for the study. The set up was arranged as indicated in Figure 1.

Separate setup was maintained with un-inoculated MSM supplemented with LDPE powder. After the stipulated time [48 h] the KOH solution [1 M] that had trapped the CO_2 liberated by the inoculant [after utilization of LDPE the sole carbon source] was gravimetrically quantified using barium chloride. The dissolved carbon dioxide present in the medium was also estimated using titration method. Briefly, sample (25 ml) was taken in a conical flask and 0.05 ml of 0.1 N Thiosulphate solution was added. After the addition of 2 drops of methyl orange indicator, this solution was titrated against 0.02 Sodium Hydroxide solution. End point was the change in color from orange red to yellow. Following this, two drops

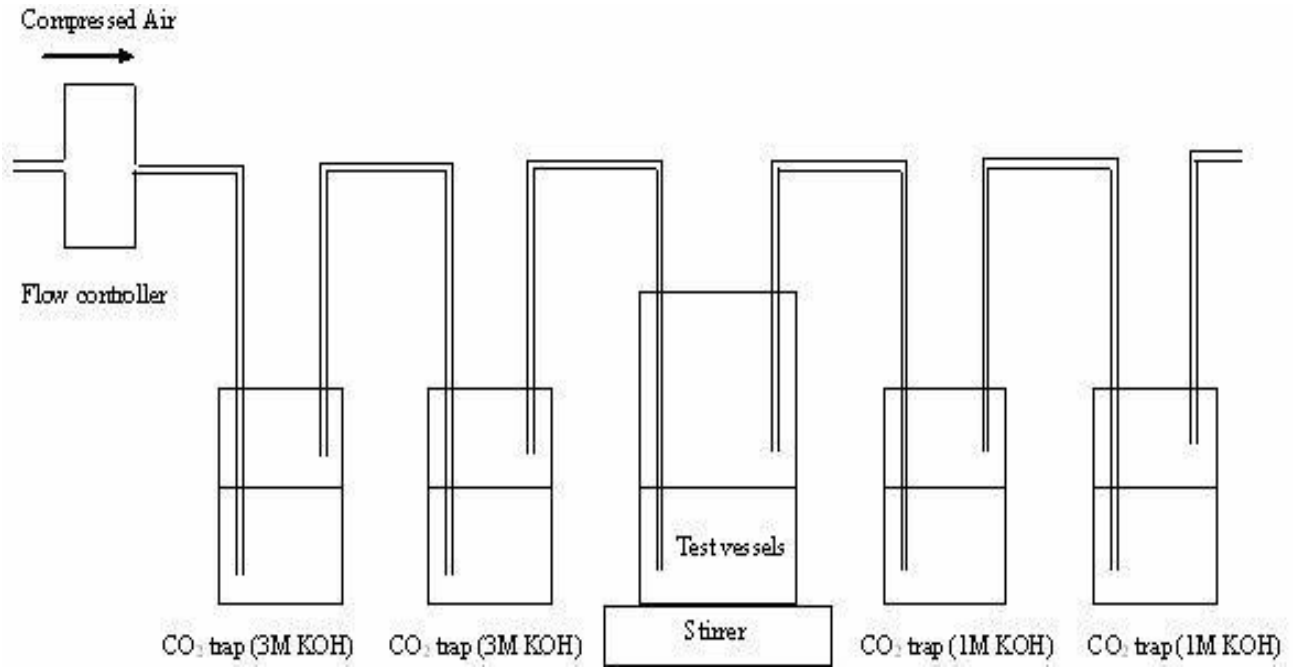

Figure 1. Sturm test.

of phenolphthalein indicator were added and titration continued till a pink color developed. Volumes of the titrant used were noted and the amount of CO_2 calculated using the formula:

$$\frac{A \times B \times 50 \times 1000}{V}$$

Where

A= ml of NaOH titrant
B= normality of NaOH
V= ml of the sample

Separate quantification was performed for test as well as control. Fungi that were used for the study were subjected to detailed macroscopic and microscopic analysis to prove their identity. This was done by growing the fungi in a slide culture.

The fungus colonized LDPE films were used for scanning electron microscope (SEM) analysis. The physical properties such as surface changes, micro-cracks, pits and holes on LDPE film by the growing fungi were analyzed by SEM.

RESULTS

Two fungal isolates SB and SD were observed to grow in the medium supplemented with paraffin wax and subsequently in the medium supplemented with LDPE powder. These isolates were further studied for colonization.

From the colonization studies, there was an increase in the fresh weight of the fungal isolates observed after 7 and 17 days of the experiments (Table 1, Table 2 and Figures 2 and 3). There was considerable increase in the weight of the isolate SB whereas there was only a substantial increase in the weight of the isolate SD.

Table 3 shows the value for CO_2 evolution from the degradation of LDPE sample by the fungal isolates. The isolate SD was found to evolve about 4 g^{-L} of CO_2 in one week and isolate SB evolved around 3.8g^{-L} of CO_2. LDPE films colonized by fungal strains were observed under SEM. Structural changes such as formation of pits, cracks and minute holes, reproductive structures and spores grown through the LDPE films were observed (Plates 1-3).

From the slide culture technique based on the microscopic morphology, we identified the isolate, SB as *Aspergillus versicolor* and SD as *Aspergillus* sp (Figures 4 and 5).

DISCUSSION

The list of pollutants which pose environmental and health hazard and are tough for biodegradation is a long one and includes solvents, wood preservative chemicals, pesticides, synthetic fibres, plastics, polyethylene etc The present study deals with the isolation of plastic degrading fungi from the marine water samples. Low density polyethylene strips were used for the study. Fungi that degraded paraffin wax were isolated first and their potential to grow in medium supplemented with LDPE as

Table 1. Colonization studies on LDPE films.

Fungal isolate	7 days (weight in g)	17 days (weight in g)
SB	0.001	0.023
SD	0.014	0.018

Table 2. Quantification of carbon dioxide evolution after degradation.

Fungal isolate	Amount of CO_2 (1 week) – g/L
SB	3.8913
SD	4.1594

Table 3. Quantification of carbon dioxide evolution after degradation

Fungal isolate	Amount of CO_2 (1 week) – g/L
SB	3.8913
SD	4.1594

Figure 2. LDPE film showing colonization of hypae.

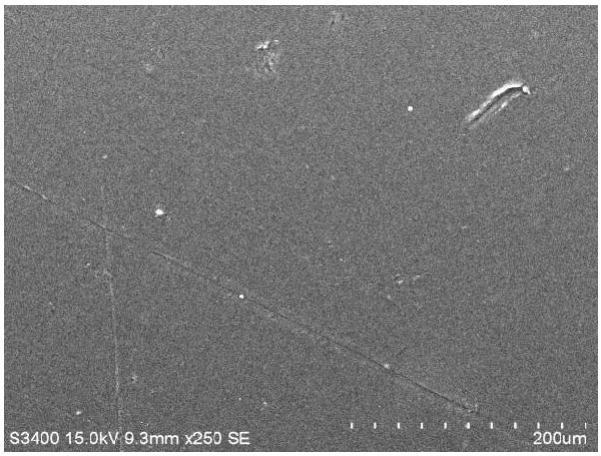

Plate 1. Control untreated LDPE film.

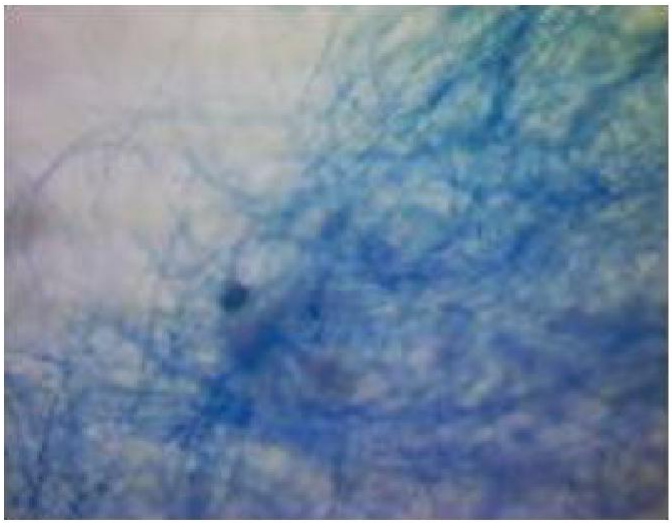

Figure 3. Microscopic view of stained fungal hyphae colonizing the LDPE film.

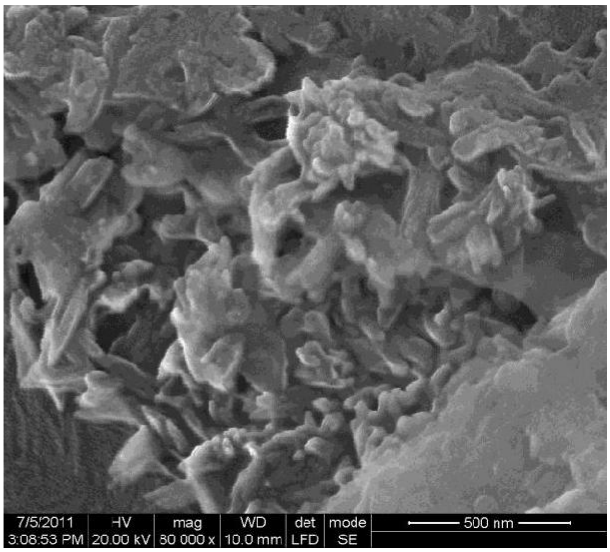

Plate 2. LDPE treated with SB.

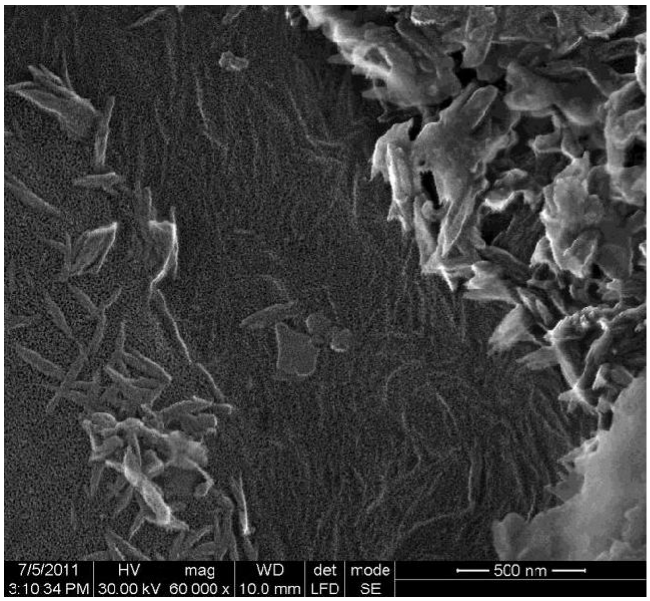

Plate 3. LDPE film treated with SD.

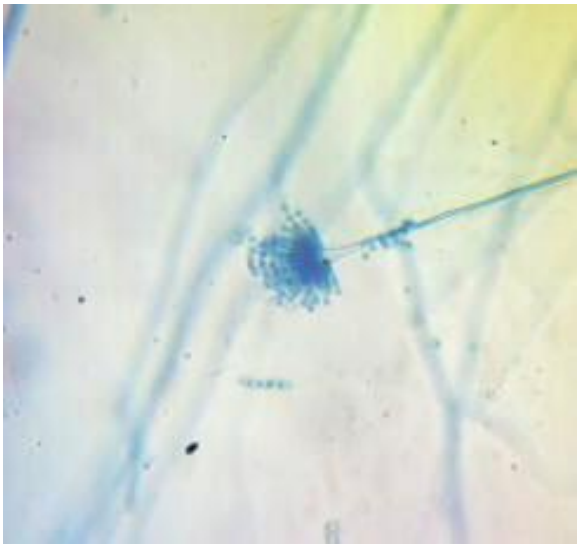

Figure 4. Microscopic morphology of isolate SB.

sole carbon source was assayed thereafter so as to study their biodegradation potential of LDPE. According to Hiroyuki et al. (1978), it is anticipated that synthetic oligomer assimilating bacteria can also degrade corresponding polymers. According to them, paraffin wax which contained most abundant species of C_{28}-C_{32} can be regarded as lower homologues of polyethylene Hadad et al. (2005) had isolated polyethylene degrading bacteria only from a group of bacterial isolates that had been

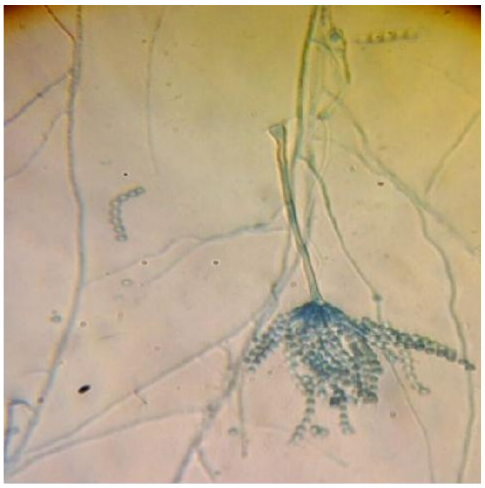

Figure 5. Microscopic morphology of isolate SD.

initially screened for utilization of a mixture of liquid waxes.

Colonization studies with the fungi yielded mediocre results with only marginal increase in the fresh weight of fungi after 7^{th} and 17^{th} day. The CO_2 evolution test gave a valid data about the degradation rate. Isolate SB showed a good degradation rate of 77% followed by isolate SD that had a degradation rate of 83%, thus proving its efficiency as the biodegrading agent.

We obtained very good results from the CO_2 evolution test (Sturm test) when compared with the studies done by Muhammad et al. (2009) who reported a concentration of about 10 g/L of carbon dioxide after a period of 30 days. Aamer et al. (2009) also reported carbon dioxide concen-tration of about 1.85 g/L after a 30 day period of growth of a fungal strain of *Fusarium* sp. on LDPE films. All these reports do not carry the initial weight of the LDPE supplied in the medium; nonetheless, the values obtained by us are far more than those that are reported. These findings corroborate the fact that even though coloni-zation of the surface by microbe is an essential factor for metabolism of the substratum, it is not necessarily correlated with biodegradation efficiency (Hadad et al., 2005).

We have done some preliminary experiments to prove that LDPE can be biodegradable if the right microorganism is isolated. We have proved that the hydrophobic LDPE film can act as a substratum for some groups of microorganisms which formed a biofilm on the LDPE film. The isolates also grew on minimal medium containing only LDPE in the powdered form as the carbon source even without any nitrogen source. We have also proved that the LDPE can be totally degraded into carbon dioxide which brings us closer to the fulfillment of the objective of isolating a microorganism

that can completely degrade the recalcitrant polyethylene if the right conditions are provided.

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