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Methods used in Biodegradation of Low Density Polyethylene (LDPE) by Fungi: A Review

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Abstract: ***LDPE (low density polyethylene) are plastic material that we use in our day to day life for various purposes.It plays a major role contributing to the pollution in the world. Many microbes, specifically fungi have been known for their degradation ability of different kinds of waste.Fungi release extracellular enzymes when they act upon a particular substrate. Researchers are trying to find out ways by which LDPE can be degraded efficiently.In this article, a broad review has been provided on methods used in LDPE biodegradation by fungi. These methods can serve as a basis for further research in this direction.***

Keywords: Biodegradation, Fungi, LDPE

1. Introduction

Biodegradation is biologically catalyzed reduction in complexity of chemical compounds[10].It is a process by which organic substances are broken down into smaller compounds by living micro organisms[11].If the biodegradation process completes fully, it is termed as mineralization.But in most cases biodegradation is usually used to describe any biologically mediated change in a substrate[12].The breakdown of the substances into smaller compounds is done by the enzymes produced by living microorganisms[13].The process of biodegradation may vary but frequently the end product is carbon dioxide or methane. Several micro organisms like fungi, bacteria and yeast are involved in biodegradation process whereas algae and protozoans reports are scanty regarding their involvement in biodegradation[14].

Low density polyethylene has long been known as the most durable and flexible plastics. These are used as carrier bags, flimsy agricultural films, laundry bags, grocery bags etc. Because they are easy to handle and are low cost,these are often used at an extensive level in the market. These are recyclable to some extent but usually are not recycled efficiently.Most of it just gets dumped in the landfill as a household or commercial or medical waste. LDPE doesnot get degraded easily. So in recent years researchers are finding a way out by which LDPE can be degraded that too biologically by using micro organisms like Fungi, bacteria and alike.Fungi especially have been studied for its LDPE degrading ability.

Fungi are achlorophyllous, ubiquitous organism and are heterotrophic capable of growing on almost any kind of substrate.They have long been known for spoiling on whatsoever it grows. These are excellent decomposers. Fungi release enzymes extracellularly. They act upon the surface of the substrate splitting it into fragments leading to minerlization releasing CO_2, H_20 and water.Till now in recent years many researchers have worked on fungal biodegradation of LDPE and reports have been obtained related to it.Since fungi play an important role in the ecosystem in the process of biodegradation,in this review an insight has been given towards methods used for fungal biodegradation of LDPE which can be useful for further studies.

Methods used

Since various reports have been obtained regarding the methods used, in this review methods are divided mainly into five sections for the ease of understanding.These five sections are Source of LDPE sample, Form of LDPE used as substrate, Source of fungal samples, Isolation method and Biodegradation measurements.

1) Source of LDPE Sample:

The Low density polyethylene fresh samples are usually obtained from any of the reliable polymer industry like Sigma Aldrich Chemical Co.(Germany) [2] [9], B.N. Polymers Bangalore[7],Gwalior Plastic Industry [8] etc.

2) Form of LDPE used as substrate:

a) LDPE powder

In many cases low density polyethylene powder is prepared which is used with the media as a sole carbon source.LDPE sheets are cut into pieces and immersed in xylene. It is boiled for 15 minutes.As the xylene dissolves the LDPE sheets, the residue is to be crushed while it is warm using band gloves. The crushing can also be done in blender at 3000 rpm[7].The LDPE powder so obtained is washed with ethanol to remove the residual xylene which is allowed to evaporate to remove residual ethanol.The powder is to be dried in the hot air oven at 60^{o}C overnight.[1,3,8]. [2] have also used polythene powder but the method used by them is not specified. The LDPE powder can also be obtained directly from reliable polymer cooperation[9].

b) Pre treatment of LDPE bags:

According to other reports [5][6], polythene bags can be obtained from polymer industry and then further processing can be done. The polythene bags obtained from reliable source is cut into small pieces of 2x2cm and used along with the media as a carbon source for fungi [5].

3) Source of the fungi used

a) Collection of sample

Samples are collected either from air, water and soil. So far there are no reports on collection of air fungi in relation to LDPE biodegradation. Reports are mostly obtained related

to soil and water samples. Soil samples are collected from municipal soild waste land area at a depth of 2-3cm in an sterile container and air dried at room temperature [1] [7]. Garbage soil samples is collected at a depth of 3-5 cm from waste disposed sites dumped with polythene and plastics[2][9].Plastic bags with soil sample from similar kind of site as mentioned in [2] are collected at depth of 1-6 cm.The samples are collected in an air dried sterile container and then air dried at room temperature and stored in refrigerator for further studies[5]. Soil sample can also be collected at a depth of 5-6cm and stored at 4^{o}C[8].When fungal species is to be isolates from water, according to reports[3] showcasing marine water fungi study,Sea water sample is collected from coast of the Bay of Bengal 500m away from shore at the depth of 5m[3].

4) Isolation of Fungal Strains

Fungal strain isolations are also done by different methods by different authors which have been described below.

a) Inoculation of soil sample with LDPE powder in SM(Synthetic medium)

Soil sample collected is inoculated in sterilized synthetic medium.The synthetic medium contains the following contents in 1000 ml distilled water [1][7][8].The same synthetic medium is referred to as Mineral Salt medium(MSM)[3].The constitution of the medium is given in the Table.

Table 1: Constitution of Synthetic medium(SM,MSM)

Contents	Amount (g/1000ml)
K_2HPO_4	1g
KH_2PO4	0.2 g
NaCl	1 g
$CaCl_2.2H_2O$	0.002 g
$(NH_4)_2SO_4$	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
$FeSO_4.7H_2O$	0.01 g
Agar	15 g

To this synthetic medium, 100 mg LDPE powder is added and is incubated at room temperature for 1 week.[1][3][7][8]. The developed colonies are isolated and sub cultured to get pure colonies on Sabauraud dextrose agar [7] and Czapec's dox agar[8] and then preserved in slant at 4^{o}C [7] or 5^{o}C[8].

Colonization study:

The colonizing capacity of fungi on LDPE film is studied by growing fungi in petriplates. For this LDPE sheets are cut into small pieces of 2x2cm having similar size and weight. The pieces of LDPE are disinfected with 70% ethanol for 30 minutes, followed by transferring the pieces in sterile distilled water for 20 minutes. Five LDPE sheets[1][3][7] or six LPDE sheets[8] of same weight is placed in petriplates containing synthetic medium without yeast extract and is inoculated with 5 similar sized fungal colonies with the help of cork borer.The petriplates are incubated at room temperature and results are observed after 1 week to 28 days[1] or 1 week to 10 days[3] or after 30 days[7] or 1 to 4 week[8].

a) Isolation of micro organism associated with LDPE sample:

Some researchers have collected both the soil sample and LDPE sample from the same site and so their method follows a slightly different path. One gram of soil sample is added in 99ml sterile distilled water and is shaken and dilution is made.Pour plate method using Czapek'sdox agar is used to isolate fungi associated with the material.The plates are incubated at 28^{o}C for 7 days.The developed colonies are isolated and subcultured to obtain pure colonies which is preserved in slant at 5^{o}C.These fungal species are further inoculated with fresh polythene sample individually on the surface of medium in a petridish to ensure that the same species do degrade any LDPE sample taken.All petridishes incubated at 28^{o}C for 30 days[5].

b) Screening of isolated fungi by clear zone method[2][7][8]

LDPE powder is added to Mineral Salt medium at a concentration of 0.1%(w/v) and sonicated for 1 hour at 120 rpm.After this agar is added and autoclavedat 120^{o}C,15lbs pressure for 15 minutes[9] or 20 minutes[2].Sterilized media was cooled to 45^{o}C[9] andabout 15ml sterilized medium ispoured into each sterile petriplates[2].After the media is solidified, the isolated colonies is inoculated and then incubated at 25-30^{o}C[2] or 30-35^{o}C[9] for 2 to 4 weeks.The organisms producing zone of clearance is used for further analysis.

c) Identification of Polythene degrading Fungi:

Fungal strains are identified using standard identification techniques such as slide culture technique and LPCB(Lactophenol cotton blue) staining[1][7][3].But what key is followed has not been specified in these reports. But according other reports the identification of fungi is performed on the basis of macroscopic and microscopic examination. The fungi are identified after staining them with cotton blue by following the keys of Raper and Fennell [5][9][2][8].

5) Measurement of Biodegradation:

As the LDPE degrading fungi are isolated and after keeping at incubation as per mentioned particular period of time, the measurement of biodegradation is carried out both morphologically and biochemically to find out an extent to which biodegradation by a particular species has taken place.

a) Scanning electron microscopical analysis:
   LDPE sheets colonized by fungal strains for period of one month is analyzed by scanning electron micrographs [1][6][4].This is carried out to observe for any cracks found on the surface of LDPE.
b) CO2 evolution test(Modified sturm test):

The amount of CO2 evolved as a result of LDPE biodegradation is determined gravimetrically and volumetrically by sturm test[1]. Gravimetric analysis[1][3]:Test and control bottles containing SM supplemented with LDPE powder is prepared.Fungal strain is added in the test bottle and the sterile air is allowed to flow through 1M KOH solution containing bottles. The CO_2 free air is passed in the test bottles which are utilized by the inoculums that release CO_2 after metabolizing LDPE in the

absorption bottle.The test is performed at room temperature for a week after which the amount of CO_2 produced in absorption bottle is calculated by adding 0.1M $BaCl_2$ which forms a precipitate of barium carbonate.The CO_2 released is calculated by measuring the weight of the precipitate formed.Difference between the value of test and control is noted [1][3].Separate setup is made for uninoculated MSM(mineral salt medium) supplemented with LDPE powder[3].

Volumetric analysis [1][3]: The dissolved CO_2 present in the medium is also measured volumetrically using titration method.Sample(medium) 25ml is taken in a conical flask and 0.05ml of 0.1N Thiosulphate solution is added.2 drops of methyl orange indicator is added and titrated against 0.02M Sodium Hydroxide solution.Endpoint is the change in colour from orange red to yellow.After this 2 drops of phenolphthalein indicator is added and titration is continued till a pink colour is obtained. Volume of the titrant used is noted and the amount of CO_2 evolved is calculated using the formula:

$$Amount\ of\ CO2 = \frac{A \times B \times 50 \times 100}{V}$$

Where, A= ml of NaOH titrant
B= Normality of NaOH
V= ml of sample

b) Determination of weight loss of LDPE by liquid culture method[2][5][9]:

The weighted strips of polyethylene are aseptically transferred into MSM and then it is inoculated with polythene degrading micro organisms.Control is kept free from microbes.These are left in shakers at 30°C, 150 rpm for 2,4 and 6 months period.After the period of incubation,the strips are collected, washed with distilled water,shade dried and their final weight is noted.It is compared with the control and hence difference is noted[2]. In other reports, polythene pieces is recovered after 30 days of incubation period from culture medium and washed with methanoland then washed with distilled water and dried at room temperature for 12 hours and final weight is taken[4][8]. Whereas as per some other report [9], the polythene strips of size 5cmx2cm is prepared and disinfected with ethanol and air dried.These disinfected strip are added in conical flask containing 100ml MSM and inoculated with polythene degrading fungi along with a control to ensure weight difference. The flaskis kept at 30°C,120 rpm for 6 months.After which the strips are recovered and dried weight is noted.The weight loss is calculated and compared based on the formula:

$$Weight\ loss(\%) = \frac{(Initial\ weight - Final\ weight) \times 100}{Initial\ weight}$$

2. Conclusion

Low density polyethylene needs to be degraded biologically because other than that it is causing harm to the environment in way or other. Not much research has been done in this field but researches are going on in these lines simultaneously across the globe. So far the methods used by various researchers for LDPE biodegradation by fungi, have been put altogether in this review which can become handy to future researchers.

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