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BIODEGRADATION OF LOW DENSITY POLYETHYLENE BY BACTERIA FROM GARBAGE SOIL IN MUTHUPET

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ABSTRACT

Plastic have been widely used as a packing material in the form of Low density A (LDPE) Continuous accumulation of plastic in the environment can cause threat to humanity and environment. In order to stop the accumulation of plastic and to make the surroundings free from plastic, microbes are isolated from muthupet areas Garbage soil. These microbes are screened by clear zone technique using polythene cover to confirm the degradation activity. Biodegradation of polythene cover and straw (Polypropylene and poly styrene) by the isolated organisms and its makes physical and structural changes over a period of time after microbial adhesion to the granules. To check the efficiency of biodegradation, weight method was performed under laboratory conditions for 15, 30, and 45 days. Experimental data revealed that Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae. Enzyme responsible for polyethylene degradation were screened from Bacillus subtilis. Enzymes were identified as Amylase. These enzymes were produced in large amount, enzyme activity was calculated using spectrophotometric method. By observing these results we can conclude that, Bacillus subtilis may act as solution for the problem caused by polyethylene in nature.

KEYWORDS : Biodegradation-LDPE-Microorganisms-Garbage soil uses of Amylase production

Introduction

Plastic are advantageous as they are strong, light weight and durable. But, lack of degradability and the closing of landfill sites, as well as growing water and land pollution problems have led to concern about plastics. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic has assumed increasing importance in the last few years. Biodegradation is necessary for water soluble or water immiscible polymers, because they eventually enter water streams which can neither be recycled nor incinerated (Shah *et al*.,2008).

Biodegradable plastics opened the way for new consideration of waste management strategies since these materials are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities (Hamilton.JD *et al*., 1995). A number of biodegradable polyesters, namely polyhydroxyalkonoates (PHA), polylactides, aliphatic polyester, polysaccharides and copolymer or blends of the have been developed successfully to meet specific demands in various fields and industries (Lee.SY,1999).

Polyethylene is a polymer made of long chain monomers of ethylene. It is a thermoplastic commodity mostly used for packaging. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tonnes of synthetic polymers are produced world wide each year (Shimao,2001). With such huge amounts of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. It takes thousand years for their efficient degradation. The durability, light weight, and processability of polyethylene causes it to linger in the nature for centuries and end up in landfills and/or natural water resources (Jang *et al*.,2002). Although there has always been a search for efficient disposal of polyethylene yet the biological means holds certain concern other than the chemical modes. Some possible measures employed for the purpose are biodegradation and bio recycling (Yang *et al*.,2004) However, biodegradation serves a tangible alternative.

The hazard of discarding waste plastic, so called, white pollution is becoming more and more severe. The plastic waste stream emerges from domestic, industrial and municipal refuse (Jayasekara *et al*., 2005).

Materials and methods

Isolation of LD plastic degrading soil microorganisms

The plastics sheets were taken after a period of 45 days. The sheets were placed in a nutrient agar plate for 24 hrs at 37^{0}C and the organisms were isolated from the colonies which were developed on the NA plates. The isolates were pure cultured and were maintained the NA slants for future study and usage.

Identification and characterization of LD plastic degrading microorganisms

The identification of the bacterial isolates with the ability to degrade LD was performed on the basis of macroscopic and microscopic examination and biochemical tests according to Bergey's Manual of Determinative Bacteriology (Holt *et al*.,1993). The bacterial isolates were identified macroscopically by examining colony morphology; surface pigment, colony shape, size, margin, surface on nutrient agar plates and microscopic examination including Gram's staining, to study the staining behaviour, shape and cell arrangements and granulation. Spore staining and motility test were also performed. The identified and isolated microorganisms they is carbohydrate fermentation ,citrate, TSI, catalase and oxidase. All the above tests were done as per the standard microbiological procedure.

Biodegradation of LD Plastics

The isolated *Bacillus subtilis*, *Escherichia coli* and *klebsiella pneumoniae* was inoculated in a 200 ml of MSM broth flask. The sterilized plastic LD sheets of 1x1 cm were also inoculated and incubated for 15, 30 and 45 days at 37^{0}C in the shaker-incubator. The sheets were taken for the analysis. The physical changes like change in size, weight and thickness of the LD plastic sheet was done.

Enzyme Extraction

After 48 hours of fermentation the fermented media were taken out and soaked in 20 mM phosphate buffer (PH=7.0) for 30 minutes at 4^{0}C in a rotary shaker. It was centrifuged at 8000 rpm for 15 min at 4^{0}C.After this the supernatant has been collected which is enzyme extract.

Amylase assay

Alpha amylase activity of the extract was measured by DNS method (O. H. Lowry *et al*., 1951) . In brief the reaction mixture containing 1% soluble starch,20 mM phosphate buffer (PH=7),and fermented extract was taken and incubated at 37°C for 20 minutes followed by the addition of 3,5-dinitrosalicylic acid (DNA). The amount of the reducing sugar liberated during assay was estimated by measuring colour development at 540 nm by UV-VIS spectrophotometer. IU of amylase activity is defined as the amount of enzyme that liberated micromole of maltose per minute under standard assay condition.

Protein Estimation

The protein content of the extract was determined following Lowry's method (G.L.Miller., 1959)

Result

Isolation of LD plastic degrading soil microorganisms

The isolated microorganisms from the NA Plates that was identified as, *Bacillus subtilis*, *Escherichia coli and Klebsiella pneumoniae*. The identification was done based on the morphological and physiological characteristics of the organism. The results for the characterization tests are as shown in table 1.

Table 1. The identification and characterization of id plastic degrading bacteria

TEST	IDENTIFIED MICROORGANISMS		
	Bacillus subtilis	***Escherichia coli***	Klebsiella pneumoniae
Size	Rod	Rod	Rod
Shape	Pair chain	Single	Single
Motility	+	+	-
Gram Staining	+	-	-
Glucose	+	+	+
Sucrose	+	+	+
Colour	white-tan	yellow	Light blue
Lactose	-	+	+
TSI	-	-	-
Citrate	+	-	+
Oxidase	-	+	+

Table 2.Change in the physical properties of the plastic during biodegradation

Name of the Bacteria	Initial weight Plastics	Number of Day		
		15	30	45
Bacillus subtilis	1000mg	0.985	0.935	0.812
Escherichia coli	1000mg	0.990	0.942	0.875
Klebsiella pneumoniae	1000mg	0.989	0.965	0.845

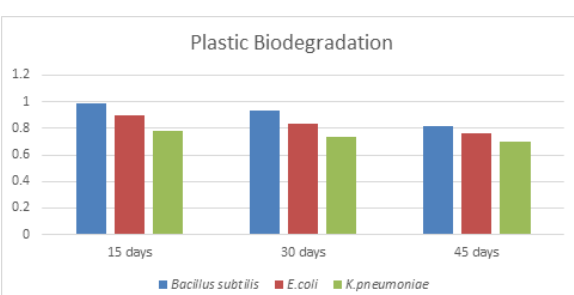


Table 3. Effect of temperature on specific activity of Amylase production from *Bacillus subtilis*.

Different Temperature	**25**°C	**30**°C	**35**°C	**40**°C	**45**°C	**50**°C	**55**°C
Enzyme Assay	0.148	0.238	0.376	0.317	0.291	0.186	0.160

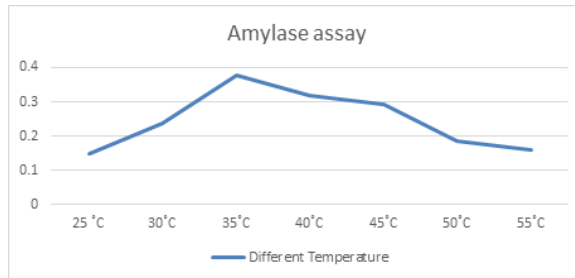
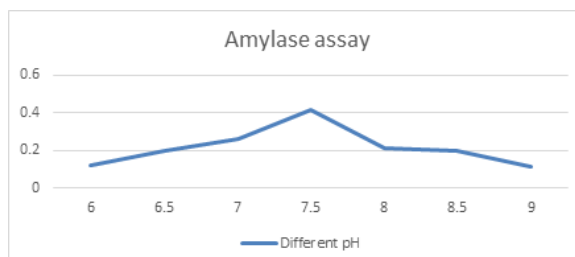


Table 4. Effect of pH on specific activity of Amylase production from *Bacillus subtilis*.

PH	6	6.5	7	7.5	8	8.5	9
Enzyme Assay	0.123	0.198	0.263	0.415	0.312	0.296	0.216



Discussion

The result were compared with earlier research studies done by Vijaya&Reddy (2008) in which they reported the average number of heterotrophic bacteria found in association with polythene film and plastic cup were $37.08x10^4$and $38.04x10^4$,$26.94x10^2$ and $35.13x10^2$ respectively Kathiresan (2003). In present study of the plastic materials in Garbage Soil are rich total heterotrophic bacterial counts $76.67x10^3$and the plastic material have colonized commonly by three species of bacteria (*Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*. In this manner results of the study are in conformity with these previous findings and similar microbes were reported during the study Ishigaka *et al*,. (2000) reported that the abundance of polymer degrading microorganisms were in seabed solid waste disposal site. Similarly , Imam *et al* (1999) observed that significant biodegradation of plastic can be occurred only after colonization by resident microbial populations and he conclude that an increase in the bacterial load has correlation with degradation of the polymer. The mechanism of biodegradation of polymer granules happened in three steps, in first step microorganism attached to the polymer granule, in second steps they grow around the granule and in last step theses microorganisms degradation the polymer and utilized it as carbon source.

Kathiresan and Bingham (2001) reported that biodegradation of polytheneby bacteria was 2.19 to 20.54% for polythene by bacteria was 2.19 to 20.54% for polythene and 0.56 to 8.16% for polythene and 0.56 to 8.16% for plastics. The present study of biodegradation of polythene by bacteria was 1000mg to 93.5% for polythene are 81.2% for plastics. Among the species *Bacillus subtilis* highest degradation 81.2%. Among all the species *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* more active the *Bacillus subtilis* in degrading 96% of polythene 81.2% of plastics with in a month. Singh *et al* (2012) reported that *Penicillium* was more active in reducing LDPE i.eupto 6.58% compared to A. fumigates as it reduced the weight upto 4.65%.

The degradation is due to extracellular enzymes release by the organisms. The breakdown products of polymer should be completely utilized by microorganisms as carbon source to control environmental

pollution Narayan(2006). Furthermore it is clearly reported by Oskay *et al*.(2004). *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* are the most widely distributed group of microorganisms in nature which primarily inhabit the soil.

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