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Research Article

BIODEGRADATION OF POLYTHENES BY BACTERIA ISOLATED FROM SOIL

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ABSTRACT

Polythene plays an important role in packaging of goods, food material, medicine and garbage bags etc but its degradation is becoming a great threat and vital cause of environmental pollution. There are various polythene degradation methods available but the eco-friendly and acceptable method is by using microbes. The present study deals with the isolation, identification, screening and degradation of pretreated polythene by microorganisms obtained from soil. A total of 15 bacteria were recovered from different areas. Further Screening of polythene degrading microorganism was done by zone of clearance method out of 15 bacteria only 3 showed the positive results and identified to be Staphylococcus sp (P1A), Pseudomonas sp. (P1B), and Bacillus sp. (P1C). A total of three isolates P1A, P1B, PIC and one Consortium PID(P1A+P1B+P1C) were used for degradation of 10 and 40 micron polythene. Bacillus sp. (PIC) showed 42.5% followed by Staphylococcus sp. (P1A) 20% Pseudomonas sp. (P1B) 7.5 % and consortium (PID) 5% degradation by weight loss in 40 days. in 40 micron polythene. We can conclude that Bacillus sp may act as solution for the problem caused by polythene in nature. Hence from this study it can be speculated that microbes has enough potential to degrade plastic with due course of time.

Keywords: Consortium, Degradation, Environmental pollution and Polythene.

INTRODUCTION

Plastics can be said as an building materials as they are being used for various purposes in our daily life (Gnanavel et al.,2012). On the contrary they causes the environmental pollution by getting accumulated in the environment this takes place because of their stable nature (Hemashenpagam et al.,2013;). In most of the countries this plastic pollution are caused due to improper recycling and waste management systems (Jayasiri et al, 2013). Biodegradation is a process which include microorganisms like bacteria and fungi that can degrade the polythene and therefore the process of Biodegradation is an upcoming trend in this field of degradation (Gu et al,2000). The microbial degradation of plastic is carried out by enzymatic activities which leads to the breakdown of polymer into monomers and oligomers followed by metabolism by microbial cells. Aerobic metabolism leads to the production of carbon dioxideand water (Starnecker and Menner,1996) and on the contrary anaerobic metabolism production of carbon dioxide, water and methane as the end products (Gu et al.,2000).

Worldwide utilization of polyethylene is increasing at a rate of 12% per annum and approximately 140 million tones of synthetic polymers are produced each year (Shimao, 2001). It takes thousand years for their efficient degradation. Huge amount of polythene getting accumulated in the environment, so their disposal creates a big problem in terms of ecology. Some possible methods are there for this purpose are biodegradation and biorecycling (Yang et al.,2005).

Currently enzymatic degradation is most widely used methods for plastics waste treatment. This method of

biodegradation by microbial enzymes increases the rate of degradation of plastics without causing any harm to the environment (Bhardwaj et al, 2012).

Polythenes in large amount get accumulated in the environment and thus create environmental issue. It is necessary to degrade polythene from atmosphere therefore an attempt has been made in this paper to isolate those microorganism that degrades the polythene.

MATERIALS AND METHODS

Sample collection: Soil samples were collected from different areas of Dehradun and brought to the laboratory, preserved under laboratory conditions for further use. Polythene samples of different densities such as 10 micron and 40 micron were purchased from local market of Dehradun.

Isolation of bacteria

Serial dilution method

1.0 gram of soil sample was transferred into a conical flask having 99ml of sterile distilled water. The mixture was shaken and serially diluted (Cappuccino and Sherman., 1996).

Petriplate method:

Further the Isolation of microorganism were carried out by spreading the dilution and the polythene strips of 3×3cm were cut and placed on the nutrient agar plates. After the incubation the growth of microorganism were seen on the polythene strips.

Screening of polythene degrading microorganism

This was carried out by zone of clearance method where the 0.5 concentrations of PEG were used in minimal media containing salts of ammonium and potassium and the zone of clearance around the colonies were observed by staining with Coomassiee blue this indicate its capacity to utilize polythene as C-source and degrade polythene (Sowmya et al., 2014)

Characterization and identification of microorganism:

After screening the isolates were characterized by various morphological and biochemical test, according to Bergey's manual of determinative bacteriology (Holt et al., 1994).

Pre-treatment of polythene

The polyethylene bags were cut into the small strips and transferred to a fresh solution having 70 ml Tween 80, 10 ml bleach, and 983 ml distilled water and stirring for 30 to 60 minutes (El-Shafei et al., 1998). Then the strips were transferred onto a beaker with distilled water and stirred for 1 hour. Further, they were aseptically placed in the ethanol solution 70% v/v for 30 min. Finally, the polyethylene strips were transferred to a Petri dish and used to disinfect the polyethylene.

Degradation of Pre Treated Polythene

Initially weighed strips of 3×3-cm size of 10 and 40 micron polythene were aseptically transferred to the conical flask containing 50 ml of nutrient broth medium and inoculated with bacteria (0.5ml). Control was maintained with plastic discs in the microbe-free medium. Different flasks will be kept in a shaker for 10, 20, 30 and 40 days respectively. After the respective duration of shaking, the polythene strips were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight and percentage weight loss were calculated using below formula. (Usha, et al. 2011)

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

RESULTS

A total of 15 bacteria were recovered from different areas. Areas selected were petrol pump, hospital and local area.

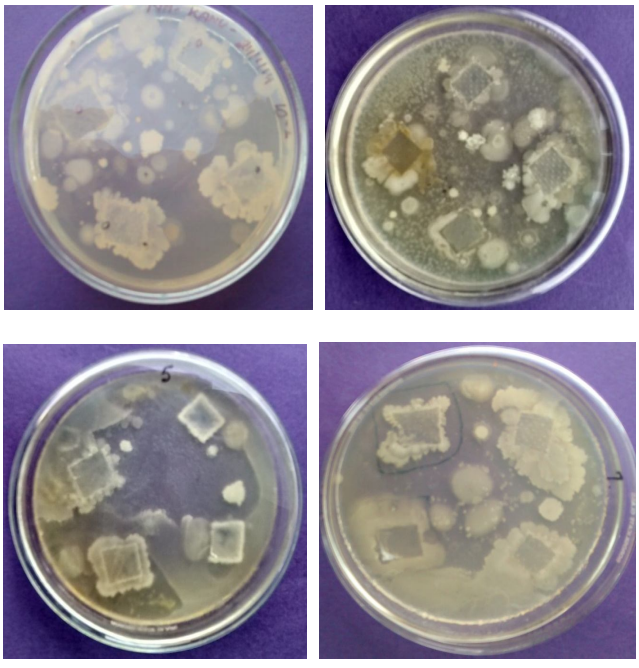

Figure 1: Preliminary Isolation of Bacteria

Screening of polythene degrading microorganism

Primary screening

In this procedure zone of clearance method was observed by staining with Coomassiee blue where the 0.5 concentrations of PEG was used in minimal media containing salts of ammonium and potassium. Out of 15 bacteria, 8 showed the positive results. Among these bacteria 5 were from petrol pump, 2 from hospital and 1 from local area.

Table 1: Primary screening of isolates obtained from different sites

S. No.	Area	Numbers of positive isolates	Zone of clearance
1.	H	2	+ +
2.	P	5	+ + +
3.	L	1	+

*Where H- Hospital, P- Petrol pump and L - Local area

+ + + - maximum clearance.

+ + - moderate clearance.

+ - minimum clearance.

Figure 2: Screening through Zone of clearance method.

Secondary screening

In this process the zone of clearance was observed by adding 1.0 concentrations of PEG followed by staining with Coomassiee blue. Out of 8 only 3 isolates of petrol pump were showed maximum clearance and were designated as: P1A, P1B and PIC.

Characterization and identification of microorganism

Microorganisms that showed positive degradation rate were identified as P1A-Staphylococcus sp., P1B-Pseudomans sp. and PIC-Bacillus sp. Various results were seen by performing staining and biochemical test. As per the table 2, it is observed that P1A is gram +ve which showed positive result for Nitrate reduction test, Methyl red and catalase and negative for Urease activity, H2S production, Citrate utilization, oxidase, Voges proskauer and Starch hydrolysis. P1B is gram –ve, which showed positive results for tests like Nitrate reduction, Citrate utilization, oxidase, catalase, and negative for Urease activity, H2S production, Voges proskauer, Methyl red, Starch hydrolysis. P1C is gram +ve which showed positive results for tests like Nitrate reduction, catalase, Starch hydrolysis and negative for Urease activity H2S production, Citrate utilization, Voges proskauer, methyl red, and oxidase.

Preparation of consortium

Consortium were prepared by mixing all three positive isolates and designated as P1D (P1A+P1B+P1C). A total of three isolates and one consortium were used for degradation of polythene.

Degradation of Pre Treated Polythene

Initially weighed polythene strips were transferred to conical flask and all the flasks were kept in shaker for respective days. After the respective duration of shaking, the polythene strips were collected and then final weigh were taken out. From the data collected weight loss of the plastics were calculated as shown in the table 3.

The above study has covered major concern on those microorganism which shows maximum as well as minimum degradation of polythene. Initially weighed, two types of polythenes (10 micron and 40 micron) were taken and kept for degradation at respective interval of days (10, 20, 30 and 40). Later on by taking out the final weighed and the degradation percent it was noted that the isolates which shows maximum degradation at 40 days of 10 and 40 micron polythene was P1C(Bacillus sp.) and the degradation percent was 26.6 % and 42.5% respectively. on the contrary the isolate which shows minimum degradation in 40 days of 10 and 40 micron polythene was P1D (P1A+P1B+P1C) and P1A (Staphylococcus sp.) along with degradation percent of 20% , 5% and 16.6% , 20% respectively.

Similarly for 30 days the isolate which shows maximum degradation of 10 and 40 micron polythene was P1C with

Table 2: Morphological and biochemical characterization of recovered isolates

S.NO.	CHARACTERISTICS	P1A	P1B	P1C
01.	Gram staining	Gram +ve	Gram -ve	Gram +ve
02.	Shape	Cocci	bacilli	bacilli
03.	Nitrate reduction	+	+	+
04.	Urease activity	-	-	-
05.	H_2S production	-	-	-
06.	Citrate utilization	-	+	-
07.	Voges proskauer	+/-	-	+/-
08.	Methyl red	+	-	-
09.	oxidase	-	+	-
10.	catalase	+	+	+
11.	Starch hydrolysis	-	-	+
	Identified isolates	Staphylococcus sp.	Pseudomonas sp.	Bacillus sp.

Table 3: Comparative analysis of polythene weight loss with different microbial species under laboratory conditions

S. No.	Name of isolates	Initial weight		10 days		20 days		30 days		40 days	
		---------- Final weight ----------									
		Polyethene types		Polyethene Types		Polyethene types		Polyethene Types		Polyethene Types	
		10 micron	40 micron	10 micron	40 micron	10 micron	40 micron	10 micron	40 micron	10 micron	40 micron
1.	P1A	0.03	0.04	0.03	0.04	0.03	0.036	0.03	0.032	0.025	0.032
2.	P1B	0.03	0.04	0.03	0.04	0.028	0.04	0.025	0.038	0.024	0.037
3.	P1C	0.03	0.04	0.027	0.03	0.026	0.038	0.023	0.028	0.022	0.023
4.	CONSORTIUM	0.03	0.04	0.03	0.04	0.027	0.04	0.025	0.041	0.024	0.038

Figure 3: Degradation of Pre Treated Polyethene

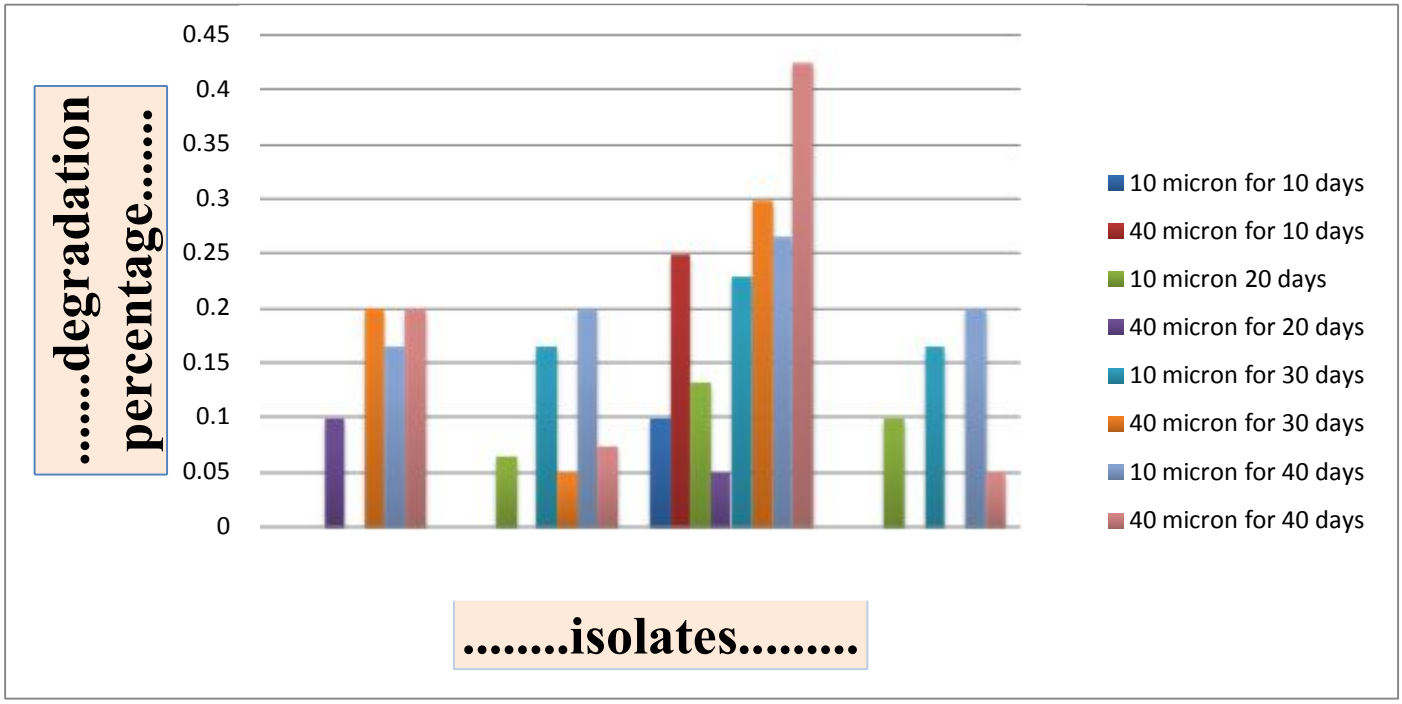

Figure 4: The degradation percentage of polythenes by different isolates

the degradation percent of 23% and 30% respectively and which shows the minimum degradation of 10 and 40 micron polythene was P1A (Staphylococcus sp.) and P1B (Pseudomonas sp.) respectively.

Maximum Degradation percentage was observed during 20 days intervals in case of isolate P1A (Staphylococcus sp.) which shows no degradation in 10 micron whereas 10% for 40 micron polythene and in case of isolate P1C (Bacillus sp.), degradation percentage for 10 micron polythene was 13.3% and 5% for 40 micron respectively and minimum degradation was shown by isolate P1C (Bacillus sp.) and P1B (Pseudomonas sp.).

During 10 day's time interval maximum degradation for 10 and 40 micron polythene was shown by P1C (Bacillus sp.) with the degradation rate of 10% and 25% respectively and no degradation was shown by isolates P1A (Staphylococcus sp.), P1B (Pseudomonas sp.) and P1D (Consortium).

DISCUSSION

Microorganisms play a vital role in biological decomposition of materials, including synthetic polymers in natural environments. In the depolymerization process two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases (Gu et al., 2000). During degradation, exo-enzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, e.g., oligomers, dimers, and monomers, and are smaller that can pass the semi-permeable outer membranes of the microbes, and then utilized as carbon and energy sources (Frazer, 1994; Hamilton et al., 1995).

In the current study, two types of polythenes were used for the observing the degradation percentage and those were high density and low density polythene of 40 micron and 10 micron respectively. The degradation percentage increases with increase in size of the polythene that is the minimum degradation is seen in the case of 10 micron polythene followed by maximum degradation by 70 micron polythene along with moderate degradation by 40 micron polythene. As per the study concern the types of polythene used was 10 and 40 micron, so in this case the maximum degradation was shown by 40 micron polythene where as 10 micron showed the minimum degradation.

As per the previous study High-density and low-density polythenes are the most commonly used synthetic plastics and they degrade slowly in natural environment, causing serious environmental problems. (Lee et al., 1991; Gu, 2003).

As per the study of All India Plastic Manufacturers' Association (AIPM) (2014), recently decided that plastic carry bags, of width below 40 micron would not be

produced, nor imported for supply to consumers to avoid pollution hazards. This is because the thickness of the bag determines the strength of the bag to break into smaller pieces. The thinner the bag is the higher is the probability of its breakdown and mixing with the soil which seriously deteriorates the soil fauna.

In the current study a total of 15 bacteria were recovered from different sites and after primary and secondary screening 3 of them showed the positive results and identified as Bacillus sp, Pseudomonas sp and Staphylococcus sp through morphological and biochemical test. Further study was continued by degradation of pretreated polythene by obtaining degradation percentage where Degradation of initially weighed pretreated polythene was done with respective intervals of time and final weighed of polythene was observed. Isolate P1C (Bacillus sp.) shows maximum degradation in 40 days, followed by P1A (Staphylococcus sp.), P1B (Pseudomonas sp.) and P1D (Consortium).

The results of this work were compared with earlier research studies done by Sowmya, et al (2014) in which they reported that Bacillus cereus was able to grow on minimal medium containing polyethylene as sole carbon source. This showed its capacity to utilize polyethylene as carbon source and to degrade polyethylene. Degradation of polyethylene was carried out by Bacillus cereus which was isolated from dumpsite soil. Further Degradation was monitored by screening which was followed by weight loss. In Screening procedure, Augusta et al. (1993), reported that the zone of clearance around the colony is due to extracellular hydrolyzing enzymes secreted by the target organism into suspended polyesters agar medium.

On the contrary, from the study of Vatsel and Anbuselvi (2014) different strains was recovered and were identified as E.coli, Staphylococcus, Pseudomonas, Klebsiella and Bacillus. The isolated microorganisms from polyethylene dumped areas can be interacted with polythene and undergo changes in mechanical properties of tensile strength, optical changes of cracking, erosion and decolorization. It is clear that natural polymers can be degraded to some extent by microbes. The biodegradation of plastics by isolated bacteria showed clear zone. It shows the initiation of biodegradation. Maximum degradation was found to be by Staphylococcus species and the minimum degradation was found to be by pseudomonas species. Staphylococcus showed 52% degradation and pseudomonas showed 11% degradation by weight loss.

By observing these results we can conclude that, Bacillus sp posses greater potential to degrade polythene when compare with other bacteria Microbes has enough potential to degrade polythene Hence this method can be used widely for biodegradation and serve as a promising tool for the elimination of polythene from the environment.

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