https://doi.org/10.48047/AFJBS.6.15.2024.10004-10013

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

BIOREMEDIATION OF LOW DENSITY POLYETHYLENE MICROPLASTICS USING INDIGENOUS BACTERIAL ISOLATES *STAPHYLOCOCCUS* SP. AND *BACILLUS* SP.

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volume 6, Issue 15, Oct 2024

Received: 05 Sep 2024

Accepted: 20 Sep 2024

Published: 15 Oct 2024

doi: 10.48047/AFJBS.6.15.2024.10004-10013

ABSTRACT

One of the most often utilized polymers, low density polyethylene (LDPE), has contaminated the environment. Plastic use has increased over the past several decades due to its versatility. The majority of the methods for managing LDPE already in use was neither ecologically nor economically sounds. The issue brought about by LDPE wastes may be resolved via LDPE biodegradation. After being separated from the polluted soil, the bacterial strains were enhanced to increase their ability to break down LDPE. Molasses was added to the polluted soil after enhanced bacterial strains were introduced. At various trial times (0, 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60 hours), the effects of various amounts of contaminated soil, such as 0, 50, 200, 350, 500, and 650 mg, were assessed. According to the findings in treatment, bacterial growth (*Staphylococcus* and *Bacillus* species) and breakdown rates were greater at 350 mg concentrations of LDPE. As a result, the bacterial strains may be used to manage LDPE wastes without contaminating the environment through the biodegradation of LDPE.

Keywords: Low density polyethylene, contaminated soil, biodegradation, *Staphylococcus* sp. and *Bacillus* sp.

INTRODUCTION

Plastics are very beneficial materials that have been utilized to further societal welfare and technological growth. Among the many different types of plastics, LDPE represents one of the synthetic polymers that utilized in commercial manufacturing the most. Because of its inherent inertness and ease of processing, LDPE finds application in a wide range of industrial settings. As a result, plastics are being produced in greater quantities and are being used more often, yet their resistance to disintegration has led to environmental pollution. The biodegradation of LDPE has been the focus of efforts since the existing methods for managing LDPE wastes are costly and environmentally harmful (Kumari and Murthy, 2013; Kumar *et al.*, 2013)

The capacity of microorganisms to affect abiotic deterioration by enzymatic, chemical, or physical means is known as biodegradation. Exo-enzymes of microorganisms degrade complex polymers into smaller molecules that may cross semi-permeable outer layer and serve as sources of energy. Because they have enzymes and may come into touch with the plastic's surface due to their tiny size, microorganisms are well suited for the biodegradation of plastic (Vatseldutt and Anbuselvi, 2014). Sarker *et al.* (2012) revealed that a few microorganisms are known to break down LDPE. With the synthesis of bio-surfactant to improve the adhesion to the surface, Bacillus sp. has demonstrated greater breakdown (up to 23% in one month) of plastic (Maniyar

et al., 2011). Plastics make up just 8–12% of the debris that ends up in landfills and has an impact on the environment. By consuming plastics, a large number of aquatic and terrestrial species perish every year. Furthermore, the productivity of agricultural land is decreased by the deposition of plastics.

More people are becoming aware of the use of effective microorganisms in polymer biodegradation. However, despite the greatest of efforts, effective bacterial decomposition of microplastics is still not attained (Harshvardhan and Jha, 2013). Because solid waste has various physicochemical and biological characteristics, screening microorganisms from landfills for trash has a large potential to reveal new bacterial strains with the ability to digest LDPE (Restrepo-Florez *et al.,* 2014; Atalia *et al.,* 2015). Therefore, a critical initial step is to screen microbes to get acceptable strains with the capacity to breakdown polyethylene. Therefore, the main focus of this work was on using indigenous bacterial isolates to bioupgrade low density polyethylene-contaminated soil.

MATERIALS AND METHODS

Sample collection

The contaminated soil was collected at Modachur, Gobichettipalayam, at the LDPE-contaminated site. The sample was labeled suitably and stored in sterilized containers in Erode District, Tamil Nadu, India, using sterile spatulas. The contaminated soil samples were mixed well. Until the experiment was carried out, the samples were kept cold.

Collection and segregation of LDPE plastic sample

Using a sterilized spatula, the LDPE plastic samples were collected from the numerous plastic dumpsites and landfills in Gobichettipalayam, Erode District, Tamil Nadu, India. Each sample was then appropriately identified and preserved in polythene bags. Low density polythene samples were acquired, and a laser particle size analyzer in a range of 100 μm to 200 μm was used to weigh and quantify the specific soil size. Xylene was used to soak shredded LDPE sample strips. This was cooked for thirty minutes after that. After dissolving the polythene granule in xylene, the residue was ground up in a mixer grinder. Any xylene that remained washed away with ethanol. It then evaporated, producing powdered polythene that was oven dried at 60°C for 12 to 15 hrs.

Isolation of indigenous bacterial isolates

From the LDPE-contaminated soil that was gathered from within and around the different plastic dumpsites and landfills in Gobichettipalayam, Erode District, Tamil Nadu, India, native bacterial strains were identified. Bacterial population in the soil sample was determined by the serial dilution techniques as the method adopted by Harley and Prescott (1993). In a facultative condition, 100 ml of nutritional medium were combined with about 1g of soil sample, and the mixture was then cultured for 24 hours. The aforementioned culture was looped out and streaked onto an agar plate. It was then cultured for a whole day at 4^{o}C in the freezer until it was needed later (Senthilkumar *et al.,* 2016).

Preparation of mineral media

$MgSO_4.7H_2O$ 1g, KH_2PO_4 2.12g, K_2HPO_4 2.12g, NaCl 2g, $CaCl_2$ 0.1g, and KNO_3 4g were all included in 1 lt of distilled water for the mineral medium. Prior to usage, all media were kept at room temperature after sterilized using autoclave for 15 minutes at 120^{o}C and 15 pounds of pressure. To keep the pH at 7±0.2, HCl or NaOH were used.

Collection of molasses

From Sakthi Sugars Factory in Erode District, Tamil Nadu, molasses was gathered. It served as a source of carbon. The soil was supplemented with molasses to encourage microbial activity. A table spoon (20g) of molasses has 58 Kcal.

Measurement of optical density (OD)

The optical density (OD) of all the samples taken from the aforementioned tests at various time spans (0, 12, 24, 36, 48, 60 and 72 hours) was determined using a UV-Visible Spectrophotometer at a wavelength of 540 nm.

Identification of bacterial isolates

Using standard microbiological methods and Bergey's handbook of systematic bacteriology, the identification of *Staphylococcus* and *Bacillus* spp. was completed (Chen *et al.*, 2007). Microscopy, cultural traits, and biochemical tests such as the Oxidase, Catalase, motility, Methyl Red, and Voges-Proskauer tests were employed in the techniques. Additionally, H_2S, indole, nitrate reduction, citrate and propionate utilization, casein, gelatin and starch hydrolysis, acid production from various carbohydrates, and growth at different pH, salinity, and temperature were all factors.

Determination of LDPE degradation potential of bacterial isolates

The previously stated composition of mineral agar medium was created in the lab, and pre-weighed pieces of LDPE were then put to it. After that, 0.1 ml of each isolate's adding overnight culture, and the combination was kept at 30°C for two months. Likewise, 50ml of mineral media was produced, and 1gm of 0.5 X 0.5 cm^2 LDPE pieces was added to the 50ml of mineral media that had been poured into the conical flasks. Subsequently, 5 milliliters of the overnight culture of bacterial isolates was added to the identical conical flasks, and they were cultured for 24 hrs at 30°C and 150 rpm. The mineral broth's pH was measured following the incubation time. The LDPE fragments were gathered, carefully cleaned with 70% ethanol, and allowed to air dry. LDPE pieces were then weighed to determine their ultimate weight. The following equation was used to compute the plastic reduction potential of bacterial isolates based on the collected data.

Degradation potential (%) = weight loss of the sample / original weight of sample x 100

RESULTS AND DISCUSSION

Bacteria isolates that degrade polyethylene (LDPE) by morphological and biochemical characterization

For isolation of bacterial isolates from soil isolated from Modachur, Gobichettipalayam in Erode District, Tamil Nadu, India contaminated with plastic waste contaminated soil. The findings suggested that the isolated bacteria were *Staphylococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. from colony, morphology, staining, and biochemical analysis (Table 1).

Measurement of optical density (OD) and reduction of LDPE

On nutrient media containing varying quantities of LDPE plastic powder (50, 200, 350, 500, and 650mg), bacteria were cultivated. OD was determined in these samples at particular time periods (0, 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 hrs). A greater proliferation of bacteria was noted between 50 mg and 350 mg of LDPE. However, there was a trend toward a decrease in the growth of bacteria at 500 and 650 mg LDPE concentrations. At 48 days, however, a greater rate of bacterial growth was noted of *Staphylococcus* sp. and *Bacillus* sp. (Fig. 1 and 2).

The pace at which LDPE microplastics degraded was also seen at all concentrations; however, up to 350 mg, the percentage of LDPE reduction was considerable, reaching a decrease of 75.13% (Table 2 and Fig. 3).

Screening of isolates for LDPE biodegradation in laboratory

The decomposition ratio of microplastic wastes is determined by the two bacterial isolates (BI 1 and BI 2) that have demonstrated better degradability for LDPE powder. It was established how well this isolate degraded polyethylene stripes. After one month of incubation with bacterial isolates, the polyethylene LDPE powder that was added to the conical flask and cultivated at 25–30^{o}C at 120rpm was collected, and weight loss was computed. The isolates (BI 1 and B2) had the greatest weight loss of powder, as indicated by the results in Table 1. Additionally, Omar Saad Jumaah (2017) employed the weight technique with distinct isolates of Bacillus and Pseudomonas to analyze polyvinyl alcohol (PVA) plastic bags.

The microorganisms with the highest capacity for degradation were Pseudomonas putida and Bacillus sp. This result was also consistent with research by Usha *et al.* (2011), which showed that exposure to Streptomyces species causes 46.16% of polythene and 35.78% of plastics to lose weight. This finding was obtained by avaricious use of three strains of *Pseudomonas putida* and *Pseudomonas aurginosa* kept in increased bacterial growth, as reported by Joiti Singh *et al.* (2015), which demonstrated the strain's inefficiency for LDPE breakdown over time. The isolates' ability to break down the plastic stripes and use them as a source of carbon and energy was what caused the growth to rise.

Rajashree and Bagde (2015) also used the spectrophotometric technique to measure PVA degradation. According to spectrophotometric test results, they showed an increase in PVA degradation over a month by isolates of *Bacillus* sp. (which showed 65% degradation) and Pseudomonas sp. (42% degradation). These bacteria grow on plastic strip surfaces, where their cell surfaces display hydrophobicity and they generate biosurfactants. Oda *et al.* (2008) found that it plays a crucial role in the formation of Polycaprolactone depolymerise, which is produced by the biofilm on the polythene surface. As a result, the bacteria *Alcaligenes faecalis* was able to identify various improved polymer biodegradation processes. The source of biosurfactant production, Streptomyces, was enhanced by Khopade *et al.* (2012).

CONCLUSION

It was previously established that LDPE resists biodegradation. However, the current study demonstrates how bacterial strains use LDPE as a carbon source and suggests that microorganisms are becoming used to hydrocarbons. Significantly high breakdown capacity predicted that the bacterial isolates would have to use LDPE as their only carbon source. Hence, the data obtained as proof that *Staphylococcus* and Bacillus species degrade LDPE suggests that these microbes can be used in further molecular investigations to achieve improved biodegradation.

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TABLE 1

THE IDENTIFICATION OF MICROORGANISMS USING COLONY MORPHOLOGICAL AND BIOCHEMICAL TESTS IN LDPE CONTAMINATED SOIL

S.No.	Test Parameters	Results	
		Bacterial Isolate 1 (BI 1)	**Bacterial Isolate 2 (BI 2)**
Selective Media Identification			
1.	Name of selective media	MSA media – positive – yellow coloured observed	*Bacillus* medium – positive
Biochemical Identification			
2.	Gram stain	Gram Positive (Cocci)	Gram Positive (Rod)
3.	Indole Test	-	-
4.	Methyl red Test	+	-
5.	Vogues Proskaur Test	+	-
6.	Citrate Test	+	+
7.	Motility Test	-	+
8.	Oxidase Test	-	-
9.	Catalase Test	+	+
10.	Urease Test	+	+
11.	Nitrate Test	+	+
12.	H_2S Production	-	-
13.	Starch Utilization Test	+	+
Carbohydrate Fermentation Test			
14.	Glucose Test	+	+
15.	Maltose Test	+	+
16.	Sucrose Test	+	+
17.	Mannitol Test	+	+
Identification		***Staphylococcus* sp.**	***Bacillus* sp.**

(+ Positive, - Negative, MSA – Mannitol Salt Agar)

TABLE 2

% REDUCTION OF DIFFERENT CONCENTRATIONS OF LDPE MICROPLASTICS (50, 200, 350, 500 AND 650 mg) IN NUTRIENT BROTH CONTAINING ENHANCED INDIGENOUS BACTERIAL ISOLATES AT DIFFERENT TIME INTERVALS

Duration	**Different concentrations of LDPE microplastics**				
	50 mg	**200 mg**	**350 mg**	**500 mg**	**650 mg**
0 hr	49.24	198.56	349.20	499.10	649.82
6 hrs	46.52	186.42	335.45	485.20	635.88
12 hrs	42.21	174.12	318.65	462.15	608.12
18 hrs	36.55	156.40	302.67	428.85	584.14
24 hrs	35.45	142.21	282.30	375.45	545.45
30 hrs	31.22	124.56	261.12	351.50	518.75
36 hrs	25.36	102.75	240.10	304.10	486.08
42 hrs	20.54	86.20	202.45	274.46.	452.12
48 hrs	16.75	72.15	166.21	225.67	412.15
54 hrs	8.12	58.32	115.40	195.66	388.18
60 hrs	6.25	45.65	86.24	176.24	325.25
% Reduction	**85.98%**	**76.46%**	**75.13**	**64.57%**	**49.93%**

FIG.1

OPTICAL DENSITY OF *STAPHYLOCOCCUS* SP. IN DIFFERENT CONCENTRATIONS OF LDPE CONTAMINATION SOIL DURING DIFFERENT TIME INTERVALS

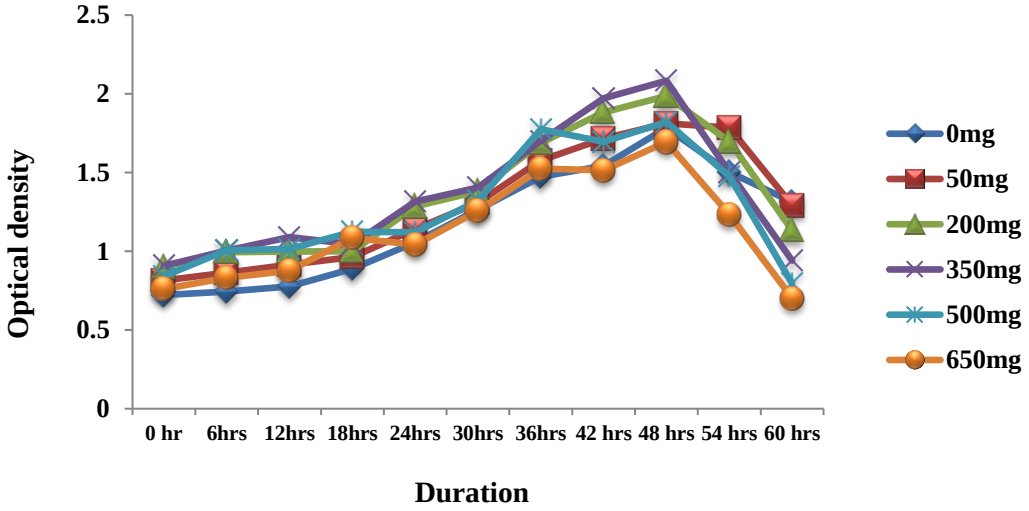

FIG.2

OPTICAL DENSITY OF *BACILLUS* SP.IN DIFFERENT CONCENTRATIONS OF LDPE CONTAMINATION SOIL DURING DIFFERENT TIME INTERVALS

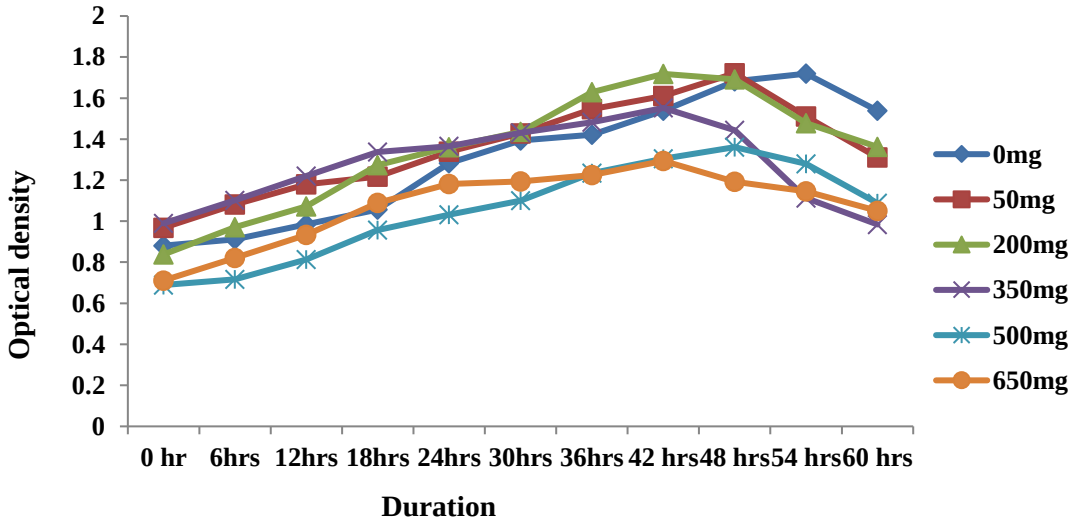

FIG. 3

% REDUCTION OF LDPE MICROPLASTICS IN VARIOUS CONCENTRATIONS (0, 50, 200, 350, 500 AND 650 mg) USING ENHANCED INDIGENOUS BACTERIA