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Biodegradation of cassava starch modified low density polyethylene by *Bacillus cereus* and *Pseudomonas aeruginosa* isolated from waste dumpsite

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Abstract

Low density polyethylene (LDPE) is used for packaging and other industrial application is a significant source of environmental pollution. The present study was aimed at testing the ability of bacterial strains identified as *Bacillus cereus* and *Pseudomonas aeruginosa* to degrade LDPE. These strains were isolated from soil samples collected from dump site. All bacterial isolates were screened for their ability to degrade synthetic LDPE. *Bacillus cereus* and *Pseudomonas aeruginosa* produced weight loss percentages of 0.18% and 0.17% respectively and were used for further studies. The biodegradation was further enhanced by blending pellets of the LDPE (90, 80 and 70 %) with cassava starch (10, 20 and 30%). The screened bacteria isolates were incubated along with the cassava starch modified LDPE for a period of 60 days. Degradation was observed in terms of weight loss and tensile strength of the modified LDPE. *Bacillus cereus* and *Pseudomonas aeruginosa* achieved a maximum weight loss reduction of 42.01 % and 51.03 % respectively in LDPE modified with 30 % cassava starch. However, the highest weight loss reduction of 54.03 % in 30 % Cassava starch modified LDPE by the bacterial consortium. Tensile strength of 42.01% was achieved in LDPE containing 30% starch. Therefore these results show that the bacteria used in this study can colonize, utilize and modify LDPE as a sole carbon source, signifying the potential of *Bacillus* and *Pseudomonas* spp. to degrade LDPE film. This work would also pave way for future studies on biodegradation to resolve the universal pollution issues.

Keywords: Low Density Polyethylene; Biodegradation; *Bacillus cereus*; *Pseudomonas aeruginosa*; Cassava Starch

1. Introduction

Low density Polyethylenes (LDPEs) are the monomers of ethylene considered as non-biodegradable in nature due to their higher molecular weight, hydrophobic backbone and chemical structure [1]. Three dimensional LDPE is a major cause of environmental pollution due to its high tensile strength, lightness, resistance to water, and microbial attack [2]. Low density Polyethylene is frequently used for wide range of purposes including, plastic bags, film wraps, lids, containers and pipe. Wastes from these plastics are one of the major problems faced by the world today. These recalcitrants are generated and accumulated in the in the environment which needs many years for complete deterioration [3]. Pollution caused by polyethylene include blockage of drainages, soil, water and air contaminations [4]. Apart from that, the three dimensional Low-density polyethene (LDPE) is a major cause of environmental pollution due to its high tensile strength, lightness, resistance to water, and microbial attack [2].

Polyethylene (as well as other plastics) remains after disposal for thousands of years in the environment. In order to manage the utility of these polymers in nature, there are two ways: one is to exploit the microorganisms in degrading polyethylene and the other is to develop artificial polymers susceptible to biodegradation [5]. Biodegradable plastics

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or bioplastics have been developed from natural products. Biopolymers currently produced from renewable resources come from Starch (cassava, corn, rice, potato), significant efforts are being made to produce biopolymer from agricultural residues/wastes. Biopolymer production from starch rich feed stocks such as corn, cassava, potato, have already been developed [6]. The long-term viability of this process is in question because it requires significantly increased amounts of arable land and this may lead to significant hike in food prices that will ultimately lead to food insecurity. The use of agricultural wastes, which is abundant in nature, will provide a cheap source of material and help in bioconversion of waste to wealth, if found suitable. Compounding petroleum based polymers with natural polymers such as starch, cellulose, lignin, chitin and chitosan is a significant way to accelerate polymer biodegradation [7].

Biodegradation is a complex process that involves the decomposition of substances by microbial activity through biodeterioration, depolymerization, assimilation, and mineralization. Low-density polyethylene (LDPE) can be degraded by chemical, photo and biological degradation [8, 9].

Various researchers have reported polyethylene degrading bacteria (*Bacillus*, *Pseudomonas, Streptococcus, Staphylococcus, Micrococcus* species) and fungi *(Aspergillus niger, Aspergillus glaucus, Aureobasidium sp. Fusarium sp. Trichoderma* sp.) [10, 11, 12]. Microbial enzymes are known to enhance the rate of biodegradation of LDPE very effectively [13]. The extracellular enzymes are considered to be too large to penetrate deeply into the polymer material and as a result, they act only on the surface of the polymer surface, thereby making biodegradation of plastics a surface erosion process [9, 14]. An attempt was made in this work to study the biodegradation of cassava starch modified LDPE by two species of Bacteria isolated from dump site. The extent of biodegradation was evaluated by comparing the initial and final dry weights of the LDPE, before and after incubation. Changes in tensile strength were further used to confirm biodegradation of the modified LDPE films.

2. Material and methods

2.1. Sample collection

Soil samples were collected from a depth of 10 cm from Ribadu hostel dumpsites within Ahmadu Bello University, Zaria, Samaru campus. These were collected with trowel and transferred into clean polythene bags and transported to Microbiology laboratory A.B.U, Zaria for further use.

The LDPE pellets and cassava starch used in this study were collected were purchased from commercial sellers in Samaru market, Zaria Nigeria.

2.2. Isolation of *Bacillus* and *Pseudomonas* Species from the soil sample:

Using an electronic weighing balance, twenty five gram (25g) of the soil sample collected was transferred into a five hundred (500) Erlenmeyer flask containing two hundred and twenty five millilitre (225ml) of sterile distilled water. The flask was shaken vigorously and allowed to stand for 30mins. One millilitre (1ml) of this solution was transferred into nine milliliter (9ml) of sterile distilled water in a test tube using a sterile two millilitre (2ml) pipette to obtain a tenfold dilution of 10^{-1}. Further serial dilutions were carried out until a dilution of 10^{-6} was obtained. Using sterile One millilitre (1ml) pipettes, zero point one millilitre (0.1ml) of each dilution 10^{-3}-10^{-6} was aseptically transferred onto the surfaces of solidified Mannitol Egg Yolk Polymyxin (MYP) Agar and Centrimide Agar, for the isolation of *Bacillus* and *Pseudomonas* species respectively; and spread well using a sterile bent glass rod. Plates were prepared in triplicates. The inoculated plates were incubated at 37 ^{o}C and observed for bacterial growth after twenty four (24) hours incubation. All growths were observed and records of colony colour and shape was kept.

Different colonies observed on both MYP Agar and Centrimide Agar plates were purified by repeated streaking of each distinct colony on Nutrient Agar (NA) plate until pure colonies were obtained. Purified bacterial isolates were transferred onto sterile NA slants and stored in the refrigerator at 4 ^{o}C for future use.

2.3. Characterization of the Bacterial isolates

Isolates were identified using the identification scheme provided in Bergey's Manual of Determinative Bacteriology (1997) based on various staining characteristics and biochemical reactions such as Gram staining, motility, oxidase and catalase tests. Finally, Microgen biochemical test-kits were used according to manufacturer's instructions to identify the isolates to species level [15].

2.4. Screening of Bacillus species for polythene degradation potential

2.4.1. Screening of Pseudomonas and Bacillus species for polythene degradation potential

Pseudomonas and *Bacillus* species being screened for their ability to utilise polythene as their sole source of carbon and energy were grown on a Basal medium ($g1^{-1}$ of distilled water) containing NH_2NO_3, 1.0; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; $CaCL_2.2H_2O$, 0.15; and yeast extract (Difco), 0.1; and 1.0mg 1^{-1} of each of the following micro- elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and $MgSO_4$ without any C- source [16]. One hundred millilitres (100ml) of the basal medium was dispensed into 250ml Erlenmeyer flasks and sterilized by autoclaving at 121^0C for 15 minutes at 15psi.

The Low Density Polyethylene pellets were thoroughly mixed with the filler and melt blended at 150^0C using a two roll mill N0. 5183 (Reliable, Rubber and plastic manufacturing company, North Bergen, New Jersey, USA). The blend was wrapped in aluminum foil and then compressed at a pressure of 3tons for 3mins at 130^0C with a hydraulic press (compression molding machine), model 3851-0. Carver Inc. (USA), flat sheets were obtained, this was done at the Polymer Laboratory, National Institute for Leather and Science Technology, (NILEST), Samaru-Zaria. The sheets were cut into thin strips, dried overnight in an oven at 60^0C and disinfected for 30mins in 70% ethanol. The strips were aseptically transferred into Erlenmeyer flasks (250ml) containing 100ml of basal medium. Cell population of1.5×10^8 CFU/ml of *Pseudomonas* and *Bacillus* species (corresponding to McFarland 0.5) were added to the flasks and labeled appropriately. All flasks were prepared in duplicates. Control was maintained in a microbe free medium. All flasks were placed in an orbital shaker at 30^0C for 30days at 150revolutions per minute (rpm) [16].

After 30 days of incubation, the Polythene strips were removed from each flask, washed with a 2 % aqueous sodium dodecyl sulphate and then rinsed with distilled water to wash off any bacterial biofilm. The strips were then placed on filter paper and dried in an oven overnight at 60^0C, and weighed thereafter using an electronic weighing balance. Resulting weights were recorded accordingly.

2.5. Preparation of the Starch-Polyethylene Composite:

Cassava starch was wrapped with aluminum foil paper and sterilized by autoclaving at 121^0C for 15mins at 15psi in order to destroy the inherent microorganisms in the starch. The cassava starch was blended together with LDPE as follows; The LDPE was thoroughly mixed with the cassava starch and melt-blended at 150^0C using a two roll mill N0. 5183 (Reliable Rubber and Plastics Manufacturing Company, north Bengen, New Jersey, USA). The blend was wrapped in aluminium foil and subjected to a 3ton pressure for 3mins on a Hydraulic press machine (Carver Inc. (USA) Model 3851-0). This was done at 130^0C using 150 × 150 moulds at the Polymer Laboratory, National Institute for Leather and Science Technology, (NILEST), Samaru-Zaria. The resulting flat sheets of composites were labelled accordingly. Four levels of filler loadings were prepared as follows;

- Pure polyethylene (PE+0%).
- Polyethylene containing 10% starch (PE+10% starch).
- Polyethylene containing 20% starch (PE+20% starch).
- Polyethylene containing 30% starch (PE+30% starch).

Polyethylene films were cut into 15 x 2 cm dumbell shapes of 1.5mm thickness. The samples were left at room temperature and weighed with a precision balance. Average weight of each polyethylene sample was recorded.

2.6. Microbial degradation of Low Density Polythene samples

The Pre-weighed plastic films were aseptically transferred into Erlenmeyer flasks containing 300 ml of nutrient broth, inoculated with *Bacillus* and *Pseudomonas* species separately. Another set up comprising of both *Pseudomonas* and *Bacillus* species (consortium) were prepared and inoculated alongside. Control was maintained with only the plastic films in a microbe-free medium. The experimental design comprised of three treatments each i.e. T1 (*Pseudomonas* + Polythene films), T2 (*Bacillus* + Polythene films) and T3 (*Pseudomonas* + *Bacillus* + Polythene films). Triplicate flasks were maintained for each treatment and left in an orbital shaker at 150 rev per min at 35^0C for 60 days. After 60 days, the PE films were collected from the flasks, washed with distilled water, shade dried and weighed, corresponding weights were recorded accordingly (Hadad *et al.,* 2005).

3. Results and discussion

3.1. Characterization of the isolates

The isolates; *Bacillus cereus* and *Bacillus licheniformis,* showed positive Gram reaction, as well as on Oxidase, motility and Arginine tests. *Bacillus cereus* showed negative for indole and ONPG tests. *Bacillus licheniformis* showed negative on indole test and positive on ONPG test (Table 1). *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* showed negative Gram reaction, Citrate and Urease tests but positive on Oxidase test. *Pseudomonas fluorescens* showed negative on ONPG test while *Pseudomonas aeruginosa* showed positive on ONPG test (Table 2).

Table 1 Characterization of *Bacillus* isolates

Test	**Isolate A**		**Isolate B**	
Growth on MYP agar	**Pink coloured colony**		**Pink coloured colony**	
Gram reaction	+		+	
Catalase	+		+	
Motility	+		+	
Arabinose	+	+	–	–
Cellebiose	+	+	–	–
Inositol	+	+	–	–
Mannitol	+	+	–	–
Mannose	+	+	–	–
Raffinose	–	+	–	–
Rhamnose	–	+	–	–
Salicin	–	–	–	–
Sorbitol	+	+	–	–
Sucrose	+	+	+	+
Trehalose	+	+	–	–
Xylose	+	+	–	–
Adonitol	–	–	–	–
Gallactose	–	–	–	–
MDM	–	–	–	–
MDG	+	–	–	–
Inulin	–	–	–	–
Melezitose	–	–	–	–
Indole	ND	–	ND	–
ONPG	+	+	–	–
Arginine	+	+	+	+
Citrate	+	+	–	–
Voges Proskauer	ND	+	ND	+
Nitrate	ND	+	ND	+
Isolate Identified	*Bacillus licheniformis*		*Bacillus cereus*	

Key: + = Positive; – = Negative; ND = Not Determined; ONPG = O-nitrophenyl-β-D-galactopyranoside; MDG = Methyl-D-Glucoside, MYP Agar = Mannitol Egg Yolk Polymyxin Agar

Table 2 Characterization of Pseudomonas isolates

Test	**Isolate (C)**	**Isolate (D)**
Growth on CA	**green coloured colonies**	**creamy coloured colony**
Gram reaction	-	-
Motility	+	+
Oxidase	+	+
Nitrate	+	+
Lysine	-	+
Ornithine	-	-
H_2S	-	-
Glucose	+	+
Mannitol	-	-
Xylose	+	+
ONPG	-	-
Indole	-	-
Urease	-	-
VP	-	-
Citrate	-	-
TDA	-	-
Gellatin	-	-
Malonate	-	+
Inositol	-	-
Sorbitol	-	-
Rhamnose	-	-
Sucrose	-	-
Lactose	-	-
Arabinose	+	+
Adonitol	-	-
Raffinose	-	-
Salicin	-	-
Arginine	+	+
Growth at 25^{o}C	+	ND
Isolate identified	*Pseudomonas fluorescens*	*Pseudomonas aeruginosa*

Key: + = Positive; – = Negative; ND = Not Determined; ONPG = O-nitrophenyl-β-D-galactopyranoside; VP = Voges Proskauer; TDA = Tyrosine-D-arginine, CA= Centrimide Agar.

3.2. Screening of the isolates for their ability to degrade LDPE

All bacterial isolates were screened for their ability to degrade low density polyethylene (LDPE). *Bacillus cereus* and *Bacillus licheniformis* produced weight loss percentage of 0.18% and 0.16% respectively Among the *Pseudomonas* spp., *Pseudomonas aeruginosa* produced the highest value of 0.17% while *Pseudomonas fluorescens* produced percentage weight loss of 0.12% (Table 3).

3.3. Biodegradation of synthetic and cassava starch modified LDPE by the screened isolates

Biodegradation result of LDPE films by the selected isolates showed that in T1 (*Pseudomonas* + Plastic films), *P. aeruginosa* achieved a maximum weight loss reduction of 51.03% in 30% Cassava starch and a minimum weight loss reduction of 3.02% in 100% LDPE (Table 4).

In T2 (*Bacillus* + LDPE films), *Bacillus cereus* achieved a maximum weight loss reduction of 42.01% with 30% cassava starch and lowest weight reduction of 1.40% in 100% LDPE (Table 5).

However, the highest weight loss reduction of 54.03% was achieved by the bacterial consortium (T3) of *Bacillus cereus* and *Pseudomonas aeruginosa* with 30% Cassava starch and lowest reduction of 3.50% in 100% LDPE (Table 6).

Table 3 Screening of bacterial isolates for LDPE degradation ability

Organism	Initial weight (g)	Final weight (g)	Weight Loss (mg)	Weight Loss (%)
B. cereus	5.0000	4.9910	9.0000	0.18
B. licheniformis	5.0000	4.9920	8.0000	0.16
P. aeruginosa	5.0000	4.9915	8.5000	0.17
P. fluorescence	5.0000	4.9940	6.0000	0.12
Control	5.0000	5.0000	0.0000	0.00

Key: LDPE- Low Density Polyethylene, Control- LDPE in basal medium without microorganisms.

Table 4 Biodegradation of pure LDPE and Modified LDPE Films Using *P. aeruginosa* (T1)

Materials	Formulation (%)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
LDPE	100	5.0000	4.8490	0.1510	3.02
LDPE/CS	90/10	5.0000	3.8885	1.1115	22.23
LDPE/CS	80/20	5.0000	3.2995	1.7005	34.01
LDPE/CS	70/30	5.0000	2.4485	2.5515	51.03
Control		5.0000	5.0000	0.0000	0.00

Key: Control- Sample films in a microbe free medium, LDPE- Low Density Polyethylene, CS- Cassava starch

Table 5 Biodegradation of Synthetic LDPE and Modified LDPE Films Using *B. cereus* (T2)

Materials	Formulation (%)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
LDPE	100	5.0000	4.9300	0.0700	1.40
LDPE/CS	90/10	5.0000	3.9495	1.0505	21.01
LDPE/CS	80/20	5.0000	3.4900	1.5100	30.20
LDPE/CS	70/30	5.0000	2.8995	2.1005	42.01
Control		5.0000	5.0000	0.0000	0.00

Key: Control- Sample films in a microbe free medium, LDPE- Low Density Polyethylene, CS- Cassava starch

Table 6 Biodegradation of synthetic LDPE and modified LDPE films using bacterial consortium (T3)

Materials	Formulation (%)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
LDPE	100	5.0000	4.8250	0.1750	3.50
LDPE/CS	90/10	5.0000	3.2850	1.7150	34.30
LDPE/CS	80/20	5.0000	2.6950	2.3050	46.10
LDPE/CS	70/30	5.0000	2.2985	2.7015	54.03
Control		5.0000	5.0000	0.0000	0.00

Key: Control- Sample films in a microbe free medium, LDPE- Low Density Polyethylene, CS- Cassava starch

The observed weight loss in synthetic and cassava modified LDPE was a result of microbial action after inoculation with *Bacillus cereus* and *Pseudomonas aeruginosa*. The degradation differs from one microbe to another because different microbe contains different characteristics [1]. Higher percentage weight loss obtained from *P. aeruginosa* for both pure LDPE and modified LDPE agrees with the findings of Tafida [17]. This may be attributed to the fact that *P. aeruginosa* has the ability to utilize utmost organic carbon for growth, as it has very simple nutritional requirements [18]. The consortium showed maximum biodegradability with over 50% degradation of modified LDPE. This showed that mixed cultures can be more useful when they grow in symbiosis, they may enhance the growth of the biofilm formed as well as increase the hydrophilicity of the polymer surface when compared to the growth of individual organisms [17]. Also, Satlewal [19] reported that microbial consortia can accelerate the rate of biodegradation of these polymers under natural environments.

It has been demonstrated that modified LDPE is not only susceptible to microbial degradation in the natural environment, but is also susceptible to macro biodegradation, that is degradation caused by organisms larger than bacteria or fungi such as insects and larger animals (Suresh *et al*., 2011). Muhonja *et al*. [20] and Gupta and Devi [1] concluded that the degradation of polyethylene led to the physical breakdown, loss of certain properties and functional groups of polyethylene which is due to the capability of microbes to secrete extracellular enzymes that can attack on LDPE.

3.4. Tensile strength analysis of the plastic samples

Change in tensile strength per cent of the plastic samples after 60 days of incubation with the bacteria is shown in Table 7. As the filler loadings increased, the tensile strength of the composites decreased more rapidly. Tensile strength is very sensitive to changes in the molar mass of polymers, which is also often taken directly as an indicator of degradation (Geweely and Ouf, 2011). Loss of tensile strength of LDPE after incubation with *Baciilus cereus* and *Pseudomonas aeruginosa* suggests that the bacteria are capable of degrading the polymer [21]. Changes in physical properties such as tensile strength determine the extent of plastic biodegradation [23].

Table 7 Change in tensile strength of the plastic samples

Materials	Formulation (%)	Initial T.S (N/mm^2)	Final T.S (N/mm^2)	Change in T.S (N/mm^2)	Change in T.S (%)
LDPE	100	9.0310	8.1279	-0.9031	10.00
LDPE/CS	90/10	7.1790	3.1903	-3.9887	55.56
LDPE/CS	20/80	5.0940	2.0345	-3.0595	60.06
LDPE/CS	70/30	4.7520	1.5815	-3.1705	66.72

Key: LDPE- Low Density Polyethylene, CS- Cassava starch, T.S- Tensile strength

4. Conclusion

On the basis of this study it can be concluded that *Bacillus cereus* and *Pseudomonas aeruginosa* indigenous to soil from dumpsite have potential for use in biodegradation of LDPE. This study would enable to develop more efficient microbial consortium having LDPE degradation capability. Moreover, to understand the mechanism of LDPE biodegradation, investigations towards the metabolic pathways and their enzymatic reactions are needed.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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