# Open Access Research Journal of Science and Technology

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(RESEARCH ARTICLE)

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## Optimum conditions for the biodegradation of waste low-density polyethylene strips by bacteria isolated from parts of north central Nigeria

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Open Access Research Journal of Science and Technology, 2023, 08(02), 001-009

Publication history: Received on 16 June 2023; revised on 01 August 2023; accepted on 04 August 2023

Article DOI: https://doi.org/10.53022/oarjst.2023.8.2.0039

#### Abstract

Optimum conditions (Temperature, pH and incubation time) were studied for selected bacterial isolates *Pseudomonas aeruginosa*1 (PA1), *Pseudomonas aeruginosa*2 (PA2), *Bacillus megaterium*, *Providencia stuarti*, *Alcaligenes faecalis*, *Enterobacter hormaechei*, *Klebsiella pneumonia* and *Proteus vulgaris* to biodegrade low-density polyethylene (LDPE) waste by using Basal Salts Medium (BSM) containing 0.500g LDPE strips (1 cm by 5 cm each) using changes in mechanical properties and weight loss of the strips as indicators for ability of these microorganisms to degrade LDPE. The results revealed that four of the eight bacterial isolates, *Pseudomonas aeruginosa*1, *Pseudomonas aeruginosa*2, *Bacillus megaterium*, and *Providencia stuarti* showed high ability to degrade the LDPE strips after 8 weeks of incubation in liquid Basal Salts Medium at pH 6.5 -7.5 and 32°C. The tensile strength of the LDPE strips was reduced by 0.047±0.41 MN/M2 (47%) for *P. aeruginosa*1, 0.040±0.30 MN/M2 (40%) for P. aeruginosa2, *Bacillus megaterium*, 0.033±0.61 MN/M2 (33%), and *Providencia stuarti*, 0.027±0.41 MN/M2 (27%). The weight loss of the LDPE recorded for *Pseudomonas aeruginosa*1 was 19.80±0.04 %, for *Pseudomonas aeruginosa*2 19.40±0.08 %, 13.40±0.10 %, for *Bacillus megaterium* and 19.20±0.42 % for *Providencia stuarti* respectively. There was significant difference in the weight loss of the LDPE strips at pH 6.5 for *Bacillus megaterium*, *Providencia stuarti*, *Alcaligenes faecalis* and *Enterobacter hormaechei* and at 30-32 °C for *Pseudomonas* spp., *Bacillus megaterium*, *Klebsiella pneumonia* and *Proteus vulgaris*(P>0.05 %).

Keywords: LDPE Biodegradation; Optimum conditions; Tensile Strength; Weight Loss

## 1. Introduction

Low Density Polyethylene (LDPE) are synthetic polymers with very high level of hydrophobicity and high molecular weight. LDPE accounts for 60% of the total plastic production and the most commonly found solid waste [1,2]. It is often used for packaging on a daily basis around the globe because of its easy processing for various products and because of its durability [3]. As carrier and grocery bags, LDPE poses a great disposal challenge because it can take up to thousand years to degrade naturally [4]. Out of 24 million tons of synthetic plastic wastes which accumulate in the environment every year, LDPE constitutes 64% of these wastes as it is used in huge quantities for the manufacture of everyday items such as bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs and water pipes. Annually, 500 billion to 1 trillion LDPE bags are being used routinely all over the world [3].

The worldwide use of polyethylene is expanding at a rate of 12% per annum [5]. Low density Polyethylene which is mostly the packaging plastic constitutes 10% of the total municipal waste generated around the globe [6]. Only a fraction of this is recycled whereas most of the wastes enter into the landfills and take years to degrade [7].

LDPE as well as other plastic wastes generated by human activity finally gets into marine water through rivers, canals/channels and municipal drainages. Beaches have also been reported to be excellent depository sites for the

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polyethylene wastes. In the marine environment, polyethylene wastes constitute a nuisance to marine life, sometimes causing blockage in the intestine of fish, birds and mammals [5].

Research has shown that these polyethylene plastics make up the highest percentage by weight (18%) of the Municipal Solid Waste (MSW) composition in Nigeria after organic wastes (57%) [8]. In this part of the world, the landfills are uncontrolled and do not conform to international standards of similar operations elsewhere [9]. Plastic waste recycling is still under-accessed in Nigeria, rather they are indiscriminately discarded in proximity to where their useful life ends abruptly; discarded on the ground, tossed out of a moving vehicle, piled up on garbage bin or stolen away by a current of wind, they immediately become an aesthetic problem and pollute the local ecosystem [5]. Increasing accumulation of polyethylene plastics in the environment has become a worldwide problem and severe threat to the planet [10]. The aim of this study was to determine the optimum conditions for biodegradation of LDPE wastes using bacteria isolated from parts of North Central Nigeria.

## 2. Material and methods

#### 2.1. Chemicals and Reagents

**Nutrient Agar** for the isolation of bacteria consisted of the following in one liter of distilled water; Peptone 10.0 g, Sodium Chloride 5.0 g, Meat extract 10.0 g and Agar 15.0 g.

#### 2.2. Nutrient Basal Media content:

The basal salts mineral media used contained the following elements (prepared in distilled water): 12.5 g/l K<sub>2</sub>HPO<sub>4</sub>; 3.8/l KH<sub>2</sub>PO<sub>4</sub>; 1.0 g/l (NH<sub>4</sub>)2SO<sub>4</sub>; 0.1 g/l MgSO<sub>4</sub>.7H<sub>2</sub>O and 5 ml trace element solution contain each of the following elements (prepared in distilled water): 0.232 g/l H<sub>3</sub>BO<sub>3</sub>; 0.174 g/l, ZnSO<sub>4</sub>.7H<sub>2</sub>O; 0.116 g/l FeSO<sub>4</sub>(NH<sub>4</sub>)2SO<sub>4</sub>.6H<sub>2</sub>O;0.096 g/l CoSO4.7H<sub>2</sub>O;0.022 g/l (NH<sub>4</sub>)6Mo7O<sub>2</sub>.4H<sub>2</sub>O; 8.0 mg/l CuSO4.5H<sub>2</sub>O; 8.0 mg/l MnSO<sub>4</sub>.4H<sub>2</sub>O.

#### 2.3. Description of the Study Area

The North Central geopolitical zone of Nigeria also called the Middle belt, consists of six states namely, Benue, Kogi, Kwara, Nasarawa, Niger, Plateau and the Federal Capital Territory (F.C.T), Abuja and the environs. This is a major division in Nigeria created during the regime of the former head of state, Ibrahim Badamasi Babangida for easy management and allocation of resources. The major towns (Capital cities) are Abuja, Minna, Lokoja, Jos, Makurdi, Lafia and Illorin. It is from these seven major towns that the three parts focused in this study were randomly drawn. Hence, Abuja, Markurdi and Jos were drawn.

#### 2.4. Isolation and characterization of bacteria

A method described by Anbuselvi, [5] was used in isolation of the bacteria. One gram of soil was transferred into a conical flask containing 99ml of sterile range solution (for easy dissolution of the sand), shaken and serially diluted. Pour plate method was used for the isolation of bacteria using nutrient agar for each dilution. The plates were incubated at 30 °C for 24hours. The developed colonies were sub-cultured repeatedly to get pure colonies and then preserved in a slant at  $4 \circ C$ .

The bacterial strains were identified macroscopically by examining colony morphology, surface pigment, shape and size on nutrient agar plates.

Microscopic examination including gram's staining was used to study the staining behavior, shape and cell arrangement.

Motility test was performed, and biochemical tests were carried out such as catalase, gelatin, hydrolysis, triple sugar, Indole, methyl red, VP, starch, and citrate tests following the Bergey's Manual of Determinative Bacteriology 2000.

#### 2.5. Pretreatment and Preparation of Low Density Polyethene Powder

The method described by Das and Kumar [2] was used. LDPE films collected from three sites randomly selected from dump sites in each of the three metropolitan cities in North Central Nigeria, were cut into small pieces (2 cm strips). Each strip was dipped in xylene and boiled for 15 minutes (until the plastic strip dissolved). It was cooled until it was palm bearable and then crushed with a blender at 3,000 rpm. This was left to evaporate the xylene, and then washed with ethanol to remove any xylene residues. It was dried in hot air oven at 60 °C overnight and stored at room temperature for further use.

## 2.6. Biodegradation Assay - Changes in Mechanical properties (Tensile strength)

The tensile strength of the polyethene strips (5 cm X 1 cm) was manually tested using a modification of the method used by Kyaw *et al.*, [11]. The maximum load at break point for each of the test LDPE strips inoculated separately with each bacterial isolate was determined ( $P_{max}$ ) and divided by the original cross-sectional area of the LDPE strip ( $A_o$ ) to obtain the Ultimate Tensile Strength (UTS) (Pmax (Newtons) /  $A(M^2)$ ). The testing conditions were maintained at a room temperature of 35-37 °C for 8 weeks with a relative humidity of 65 %. The negative control was maintained at similar incubation conditions and tested, and compared with a positive control. The test was done in triplicate and the average calculated as the final result.

#### 2.7. Biodegradation measurement- Weight loss method

The polyethylene films after exposure to each of the bacterial isolates were evaluated for weight loss using the methods of Hadad *et al.*, [12]; Kyaw and Champakalakshmi, [11]. 0.500g LDPE strips were incubated at 30°C for 2, 4,6, and 8 weeks and then washed thoroughly with 2 % (v/v) aqueous Sodium Dodecyl Sulphate (SDS) solution for 4 hours. The strips were dried at 60°C overnight in an incubator and placed on a filter paper before weighing with a microbalance; the percentage weight loss was determined using the following formula:

Weight loss (%) = initial weight – final weight/initial weight X 100.

#### 2.8. Determination of the Optimum Conditions for Biodegradation of LDPE Wastes using bacterial Isolates

#### 2.8.1. Effect of incubation Time

To determine the effect of incubation time on the ability of the bacterial isolates to degrade LDPE, Basal Salt Medium of pH 7.05 supplemented with 0.1% PE was inoculated with each of the isolates and incubated at different times, 2, 4, 6, 8 weeks [13]. Biodegradation was measured gravimetrically as percentage weight loss of the LDPE strips.

## 2.8.2. Effect of pH

The effect of pH on the ability of the predominant microbial Isolates to utilize LDPE as a sole source of carbon and nitrogen was determined using the method of Al-Jailawi *et al.*, [14]. Using a supplemented mineral salt medium (MSM) with 0.1 % LDPE at different pH values (4.5, 5.5, 6.5, 7.5 and 8.5) to determine the suitable pH. The culture was incubated in a shaker incubator (180rpm) at 30 °C for 8 weeks. The optimum pH was employed in subsequent experiment.

## 2.8.3. Effect of Temperature

To determine the effect of temperature on the ability of the microbial isolates to degrade LDPE, Mineral salt medium (with optimum pH from 3.10.1) supplemented with 0.1 % LDPE was inoculated with each of the isolates and incubated at different temperatures (28 °C, 30 °C, 32 °C, 34 °C, and 36 °C) for 8 weeks. Biodegradation was measured gravimetrically as percentage weight loss of the LDPE strips. Optimal temperature obtained for each of the isolates was subsequently employed, depending on the growth density measurement [14].

#### 2.9. Data Analysis

All analysis was conducted in triplicate and analyzed using Microsoft Excel Windows 10 program and Smith Statistical Package (SSP) version 3.1, with significance determined at 95% interval. Results are presented as means ± standard error of the mean.

## 3. Results

## 3.1. Changes in Mechanical properties (Tensile strength)

The tensile strength of biodegradable LDPE waste by bacterial isolates are as shown in Table 3.1 The change in tensile strength of the biodegraded LDPE waste observed for the bacterial isolates ranges from  $0.053\pm0.41$  to  $0.093\pm0.17$  MN/M<sup>2</sup> (7 – 47 % loss) and the highest loss in tensile strength was observed for *Pseudomonas aeruginosa* 1 (Pa1:  $0.053\pm0.41$  MN/M<sup>2</sup> (47 %)), *Pseudomonas aeruginsa* 2 (Pa2:  $0.060\pm0.30$  (40%) and *Proteus vulgaris* ( $0.060\pm0.00$  (40%) but lower for *Klebsiella pneumoniae* ( $0.093\pm0.17$  MN/M<sup>2</sup> (7 %)) and *Enterobacter hormaechei* ( $0.087\pm0.01$  MN/M<sup>2</sup> (13 %))respectively as shown in Figure 4.1

Bacteria	Tensile Strength (M			
	Cross Sectional Area(M <sup>2</sup> ) (5cm by1cm)	Force (Maximum load to break point) (MN)	Tensile Strength (Max Load to break Point / cross sectional area (MN/M <sup>2</sup> ) =	Percentage loss (%)
Control	0.15	0.015±0.00	0.100±0.00	0
Pseudomonas aeruginosa1	0.15	0.008±0.18	0.053±0.41	47
Pseudomonas aeruginosa2	0.15	0.009±0.10	0.060±0.30	40
Bacillus megaterium	0.15	0.010±0.20	0.067±0.61	33
Providencia stuarti	0.15	0.010±0.11	0.073±0.41	27
Alcalagenes faecalis	0.15	0.012±0.12	0.080±0.12	20
Entrobacter hormaechei	0.15	0.013±0.21	0.087±0.01	13
Klebsiella pneumonia	0.15	0.014±0.17	0.093±0.17	7
Proteus vulgaris	0.15	0.009±0.00	0.060±0.00	40

Table 1 Changes in the Mechanical Properties of Biodegraded LDPE Films by Bacteria

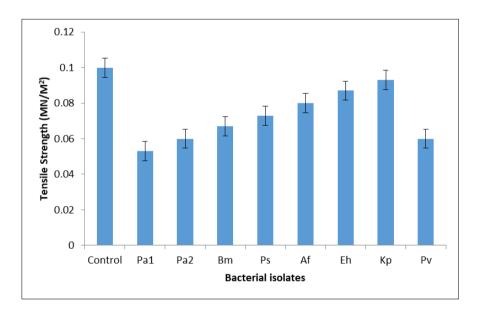


Figure 1 Changes in tensile strength of LDPE films degraded by Bacteria isolated from dump sites in parts of North Central Nigeria

#### 3.2. Optimum conditions for biodegradation - incubation period

The effect of incubation period on biodegradation of LDPE waste by bacteria isolated from soil from dump sites in parts of North Central Nigeria is as shown in tables 3.2 and Figure 3.2. The percentage weight reduction of LDPE waste by bacterial isolates between 2-8 weeks of exposure were within the range of 0.0-19.80 $\pm$ 0.04 % and the highest percentage reduction was at 8 weeks duration for *P. aeruginosa* (Pa1: 19.80 $\pm$ 0.04 %), *P. aeruginosa* (Pa2: 19.40 $\pm$ 0.08 %) and *Providencia staurti* (19.20 $\pm$ 0.42 %)but low at 2-6 weeks durations for *Klebsiella pneumoniae* with percentage weight

reductions ranging between  $0.60\pm0.17 - 1.40\pm0.02$  % and 2-8 weeks duration for *Proteus vulgaris* with percentage weight reductions ranges from  $0.0-0.80\pm0.00$  %. The differences in the weight loss of low density polythene were statistically significant between *Pseudomonas. aeruginosa* 1(Pa1) or *Pseudomonas aeruginosa* 2 (Pa 2) and *Proteus vulgaris* at 95 % interval.

Bacteria	Initial weight of LDPE strip(g)	Percentage weight loss of LDPE films over time (week (%)					
		2	4	6	8		
Control	0.500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		
Pseudomonas aeruginosa1	0.500	9.60±0.18 <sup>ba</sup>	12.80±0.41 <sub>ba</sub>	14.20±0.09 <sup>ba</sup>	19.80±0.04 <sup>ba</sup>		
Pseudomonas aeruginosa2	0.500	11.00±0.10 <sup>ba</sup>	13.20±0.30 <sub>ba</sub>	18.80±0.01 <sup>ba</sup>	19.40±0.08 <sup>ba</sup>		
Bacillus megaterium	0.500	5.40±0.20 <sup>b</sup>	11.60±0.61 <sub>ba</sub>	13.20±0.04 <sup>ba</sup>	13.40±0.10 <sup>ba</sup>		
Providencia stuarti	0.500	6.60±0.11 <sup>b</sup>	8.20±0.41 <sup>b</sup>	17.40±0.001 <sup>ba</sup>	19.20±0.42 <sup>ba</sup>		
Alcaligenes faecalis	0.500	6.20±0.12 <sup>b</sup>	6.80±0.12 <sup>b</sup>	7.60±0.21 <sup>b</sup>	8.00±0.81 <sup>b</sup>		
Enterobacter hormaechei	0.500	3.60±0.21 <sup>b</sup>	5.40±0.01 <sup>b</sup>	5.60±0.11 <sup>b</sup>	5.80±0.31 <sup>b</sup>		
Klebsiella pneumonia	0.500	$0.60 \pm 0.17^{ba}$	$1.20 \pm 0.17$ ba	1.40±0.19 <sup>b</sup>	1.40±0.02 <sup>b</sup>		
Proteus vulgaris	0.500	0.00±0.00 <sup>ba</sup>	$0.60 \pm 0.00$ ba	$0.60\pm0.00$ ba	$0.80 \pm 0.00^{ba}$		

**Table 2** Effect of Duration of Incubation on Biodegradation of LDPE by
 Bacterial Isolates

ba=significance; b=insignificance

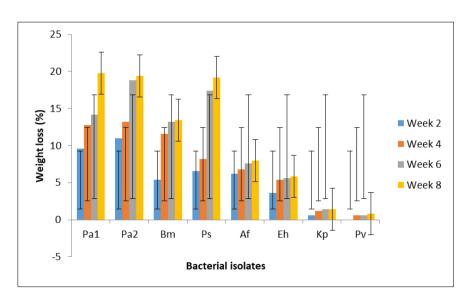


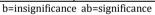
Figure 2 Percentage weight loss of waste LDPE films by Bacteria isolated from dump sites in parts of North Central Nigeria in relation to duration of incubation in weeks

## 3.3. Effect of pH on Biodegradation of Low Density Polythene (LDPE) Waste

The effect of pH on biodegradation of LDPE wastes by bacteria isolated from the soil from dump sites in parts of North Central Nigeria is as shown in table 3.3 and figure 3.3. The percentage weight reductions of LDPE in relation to pH between 4.5-8.5 by bacterial isolates ranges from 0.0 to19.80±0.11 % and the highest weight loss was at pH 7.5 for *P. aeruginosa* (Pa1: 19.80±0.11 %) and *P. aeruginosa* (Pa2: 19.40±0.04 %) but low at pH 5.5 and 4.5 for *Alcaligenes faecalis* (2.20±0.01 %) and *Enterobacter hormaechei* (2.20±0.01 %); and pH 4.5-8.5 for *Proteus vulgaris* with 0.0% weight reductions of LDPE waste as shown in Figure 3.3. There was significant biodegradation activity recorded at pH 6.5 for *Bacillus megaterium, Providencia stuarti* and *Alcaligenes faecalis* at 95 % interval.

Table 3 Effect of pH on waste LDPE on biodegradation of waste LDPE using bacteria isolates

Bacterial		pH changes – Percentage weight loss (g/g)				
	Initial weight (g/g)	4.5	5.5	6.5	7.5	8.5
Control	0.500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	$0.00 \pm 0.00$
Pseudomonas aeruginosa1	0.500	4.20±0.00 <sup>b</sup>	4.20±0.30 <sup>b</sup>	18.00±0.11b	19.40±0.04 <sup>b</sup>	8.00±0.31 <sup>b</sup>
Pseudomonas aeruginosa2	0.500	6.00±0.11 <sup>b</sup>	7.40±0.16 <sup>b</sup>	19.20±0.08 <sup>b</sup>	19.80±0.11 <sup>b</sup>	10.00±0.21 <sup>b</sup>
Bacillus megaterium	0.500	8.20±0.12 <sup>b</sup>	9.60±0.41 <sup>b</sup>	16.00±0.1 <sup>ab</sup>	19.20±0.15 <sup>b</sup>	5.40±0.14 <sup>b</sup>
Providencia stuarti	0.500	5.00±0.81 <sup>b</sup>	4.200±0.31 <sup>b</sup>	4.40±0.09 <sup>ab</sup>	4.20±0.13 <sup>b</sup>	$2.00 \pm 0.24^{b}$
Alcaligenes faecalis	0.500	2.60±0.02 <sup>b</sup>	2.20±0.01 <sup>b</sup>	12.20±0.11 <sup>ab</sup>	4.40±0.21 <sup>b</sup>	4.00±0.03 <sup>b</sup>
Enterobacter hormaechei	0.500	2.20±0.01 <sup>b</sup>	4.00±0.00 <sup>b</sup>	8.80±0.01 <sup>ab</sup>	4.00±0.00 <sup>b</sup>	2.20±0.04 <sup>b</sup>
Klebsiella pneumonia	0.500	0.00±0.0	0.00±0.00	4.00±0.03 <sup>b</sup>	4.20±0.00 <sup>b</sup>	2.20±0.00 <sup>b</sup>
Proteus vulgaris	0.500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00



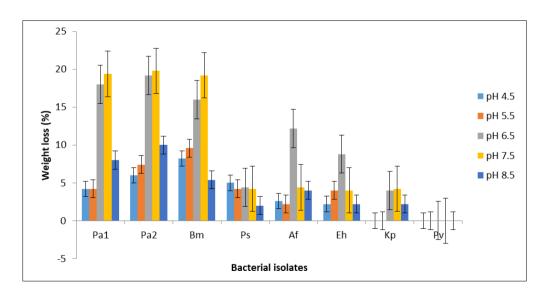


Figure 3 Percentage Weight loss of waste LDPE films by bacteria isolated from dump sites in parts of North Central Nigeria in relation to pH

#### 3.4. Effect of Temperature on Biodegradation of Low Density Polythene (LDPE) Waste

The effect of temperature on biodegradation of LDPE wastes by bacteria isolated from the soil from dump sites in parts of North Central Nigeria is as shown in Table 3.4 and Figure 3.4. The percentage weight reduction of LDPE waste by bacterial isolates at tempearture of exposure between 28 °C - 36 °C were within the range of 0.0 to  $29.4\pm0.26\%$  and the highest was recorded for *Bacllus megaterium* at  $32^{\circ}$ C,  $34^{\circ}$ C and  $30^{\circ}$ C with percentage weight reduction of LDPE waste of  $29.4\pm0.26\%$ ,  $27.6\pm0.41\%$  and  $26.2\pm0.15\%$  respectively, while *Proteus vulgaris* had the lowest percentage weight reduction of LDPE ranges between 0.0 to  $3.2\pm0.00\%$  as shown in Figure 3.4. There was significant weight loss recorded for the *Pseudomonas aeruginosa* strains as well as *Bacillus megaterium* at  $30^{\circ}$ C to  $36^{\circ}$ C at 95% interval.

Bacterial isolates	Initial weight (g)	Percentage weight loss (%) Temperature (°C)				
		28 ºC	30 ºC	32 ºC	34 ºC	36 ºC
Control	0.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	$0.00 \pm 0.00$
Pseudomonas aeruginosa1	0.50	14.2±0.01 <sup>b</sup>	24.2±0.21 <sup>ba</sup>	22.2±0.10 ba	20.2±0.14 <sup>ba</sup>	8.2±0.11 <sup>b</sup>
Pseudomonas aeruginosa2	0.50	12.2±0.14 <sup>b</sup>	22.2±0.33 <sup>ba</sup>	21.6±0.08 ba	14.4±0.11 <sup>b</sup>	4.2±0.00 ba
Bacillus megaterium	0.50	8.2±0.12 <sup>b</sup>	27.6±0.41 <sup>ba</sup>	29.4±0.26 <sup>b</sup>	26.2±0.15 <sup>ba</sup>	23.4±0.14 <sup>ba</sup>
Providencia stuarti	0.50	8.8±0.01 <sup>b</sup>	17.6±0.00 <sup>b</sup>	20.4±0.09 <sup>b</sup>	20.2±0.13 <sup>b</sup>	16.2±0.10 <sup>b</sup>
Alcaligenes faecalis	0.50	12.6±0.09 <sup>b</sup>	20.0±0.01 <sup>b</sup>	22.2±0.11 <sup>b</sup>	18.4±0.21 b	13.6±0.04 <sup>b</sup>
Enterobacter hormaechei	0.50	7.2±0.04 <sup>b</sup>	19.4±0.06 <sup>b</sup>	18.2±0.03 <sup>b</sup>	$11.4 \pm 0.00^{b}$	9.6±0.04 <sup>b</sup>
Klebsiella pneumonia	0.50	2.4±0.00 <sup>b</sup>	4.6±0.00 <sup>ba</sup>	4.0±0.01 <sup>ba</sup>	3.8±0.00 <sup>ba</sup>	3.0±0.00 ba
Proteus vulgaris	0.50	0.0±0.00	$3.0\pm0.02$ <sup>ba</sup>	$3.2\pm0.00$ ba	1.8±0.00 ba	0.0±0.00

**Table 4** Effect of temperature on the biodegradation of LDPE waste by bacteria isolates

ba=significance; b=insignificance

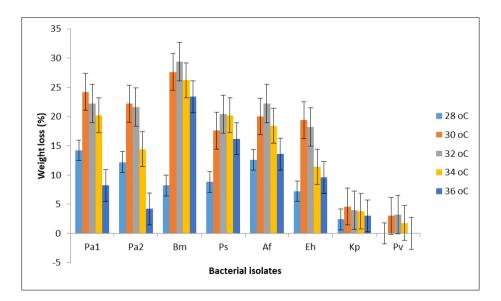


Figure 4 Percentage Weight loss of waste LDPE films by bacteria isolated from dump sites in parts of North Central Nigeria in relation to temperature

## 4. Discussion

In this study, the conditions required for the degradation of waste Low density – polyethylene strips by bacteria isolated from dump sites in some metropolitan cities in North Central Nigeria were monitored to determine optimal conditions for effective biodegradation.

The duration of incubation is an important condition that affects biodegradation processes. Results obtained in this study (table 3.2 and figure 3.2) which showed significant increase in LDPE degradation by all bacteria isolates with the time of incubation is in line with previous studies by Hussein *et al.*, [15], with LPDE degradation measured in terms of Carbon dioxide evolution. Similar results were obtained by Abdel-Shafy *et al.*, [14] for degradation measured in terms of LDPE weight loss as in this study. These results show that the hydrophobic nature of LDPE plastic polymer makes it recalcitrant in nature. For LDPE to yield to degradation, under the right environmental conditions, time is required for the microbes to grow and form biofilms on the surface of the polymer. This helps them to attach to the polymer surface and release extracellular enzymes on the polymer. Time is also required for these enzymes to permeate the polymer surface and gradually erode the surface often with the formation of pits and holes that eventually disintegrates the polymer causing degradation. The longer the period of incubation, the greater the chances of biodegradation of the LDPE polymer.

Significant increase in LDPE degradation was observed between pH 4.5 and 7.5 (Table 3) for most of the bacterial isolates with pH 7.5 identified as the most suitable pH for LDPE degradation. The gradual decline in the capacity of the bacterial isolates to utilize LDPE at pH 8.5 is similar to results obtained by Hussein *et al.*, [15] which showed that optimal pH for the growth of LPDE degrading bacteria occurred at pH 7.0 after 15 days of incubation and declined at higher pH. In another study by Esmaeli *et al.*, [16], it was also found that suitable pH for LDPE film degradation in situ by *Lynsinibacillus xylanilyticus* occurred at pH 7.5.

The gradual increase in temperature form 28 °C to 36 °C (Table 4 and Figure 4) had some significant effect on some of the bacterial isolates. LDPE degradation for most of the bacterial isolates occurred at temperatures between 30 °C and 32 °C with a gradual decline in LDPE degradation recorded as the temperature increased. Burd, [17], revealed that polyethylene (PE) film degradation significantly increased at 30 °C for six weeks for *Sphingomonas* sp. and *Psuedomonas* sp. Similarly, increasing temperature has been found to decrease growth of some bacterial isolates [18].

The gradual decrease in bacterial growth and decrease in LDPE utilization activity may be attributed to the accumulation of metabolites resulting from oxidation processes produced by bacterial isolates, or to a lack of oxygen and nutrients as suggested by Bishnoil *et al.*, [18]. This is different from results obtained by Kumari *et al.*, [19], which reported enhanced PE degradation at higher temperatures of 40 °C by 24 – 28 % compared to results obtained at 30 °C (18 – 21 %).

## 5. Conclusion

This study shows that the various bacteria isolated from this study, namely *Pseudomonas aeruginosa*1 (PA1), *Pseudomonas aeruginosa*2 (PA2), *Bacillus megaterium, Providencia stuarti, Alcaligenes faecalis, Enterobacter hormaechei, Klebsiella pneumonia* and *Proteus vulgaris* were able to degrade low-density polyethylene at pH 7.5 and temperature of about 30 °C after 8 weeks of incubation. The capacity to degrade LDPE increased in all cases with increase in the period of incubation. This assessment of the optimal conditions for LDPE degradation can be applied in commercial biodegradation. The process could also be enhanced by the pretreatment of the LDPE strips or the use of bacterial consortium in the degradation process.

## **Compliance with ethical standards**

## Disclosure of conflict of interest

The author declared no conflict of interest exist.

## Authors Contributions

This study was conducted in collaboration of all authors. All authors read and approved the final version of the manuscript.

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