



Polyethylene Biodegradation Potentials of *Pseudomonas aeruginosa* and *Micrococcus* sp. Isolated from Waste Dumps and Farmlands in Nsukka, Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author VC designed the study, wrote the research protocol, supervised the analyses and performed the statistical analysis. Authors UO and JU did all the laboratory analyses. Author CN managed the literature searches, performed further statistical analysis and wrote the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Low Density Polyethylene (LDPE) are plastic materials extensively used in packaging, constituting recalcitrant environmental pollutants that defy natural degradation processes.

Aim: This study isolated bacteria from a Nigerian environment and assessed their potential for LDPE biodegradation.

Methods: Using standard procedures, Bacteria were isolated from polythene samples collected from farmlands and waste dump sites in Nsukka metropolis. Mineral salt medium (MSM) was prepared, with LPDE as sole carbon source, and used for isolation. Optical density (OD₆₀₀ nm) was used to study bacterial growth on LDPE as sole carbon source as proof of biodegradation. Both organisms demonstrated steady growth on LDPE over time.

Results: *Pseudomonas aeruginosa* and *Micrococcus* sp. were identified based on morphological

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and biochemical characteristics. Ability to grow on LDPE as a sole carbon source was studied as evidence of polyethylene biodegradation. Organisms were inoculated into MSM and incubated at 37°C and 50°C for 15 days. Maximum growth was recorded after 15 days of incubation for both organisms. *P. aeruginosa* and *Micrococcus* sp. showed steady growth at 37°C as well as 50 °C. *Micrococcus* sp. recorded highest growth; 0.324 nm and 0.312 nm at 37°C and 50°C respectively, after 15 days. Similarly, *P. aeruginosa* recorded highest growth of 0.40 nm and 0.258 nm for 37°C and 50°C respectively. LDPE degradation increased with increase in time.

Conclusion: This study demonstrates the enormous polyethylene-degrading potentials of *P. aeruginosa* and *Micrococcus* sp. isolated from Nsukka, Nigeria.

Keywords: Low Density Polyethylene (LDPE); *P. aeruginosa*; *Micrococcus* sp.; biodegradation; contaminants.

1. INTRODUCTION

The natural environment is built to support life. Its ability to sustain life depends largely on maintaining a self-system balance. This maintenance depends largely of degradation of complex matter and subsequent cycling of nutrients and every form of matter [1]. This balance and working of the environment is often threatened by anthropogenic activities resulting to various undesirable consequences [2,3]. A notable consequence of the human activities is waste generation and consequent poor waste management [4-6]. Severally, these wastes generated defile the natural degradation processes and often upset the natural ecosystem balance.

Xenobiotics are worthy of note as recalcitrant wastes that defy the natural cycles [7]. An important class of Xenobiotics is plastics, which include polythene and other examples [8]. Production of plastics is placed at about 140 million tons yearly and up to 30% of these are used in packaging globally [9]. As expected, people use the products packaged and the packaging material end up in the ecosystem as wastes. The yearly increase in the use of Low-Density Polyethylene (LDPE) is placed at about 12% [9]. In Nigeria, this has become a great challenge based on numerous reasons. For example, the rise in popularity of LDPE for packaging has increased exponentially since the advent of the sachet water factories popularly known as "Pure water". The result is that most Nigerian cities are littered with polythene wastes with dire consequences [10,11]. The menace is worsened by lack of proper waste disposal steps and method as demonstrated in other parts of the world [11]. In Europe and America, the reduce, reuse and recycle (3Rs) policy is strongly advocated and implemented [9,12]. Bangladesh has been drastic to the point of placing a ban on

importation of plastic bags since 2002 [9]. In Africa, Kenya has also shown efforts to encourage the 3Rs policy [13].

Apparently, generation of polythene waste is unavoidable in today's world. This makes for steps towards the successful removal of this important contaminants from the environment. The recalcitrant nature of polythene is due to the hydrophobicity of its parent compounds [14]. Adekomaya and Ojo [11] has suggested the use of polythene in energy generation. Other methods of managing polythene waste have been suggested and used [15]. However, these methods are with numerous adverse effects on the ecosystem. Biodegradation is generally accepted for many advantages; ranging from cost effectiveness to no adverse effects on the ecosystem [16,17].

Biodegradation uses microorganisms, such as bacteria, to degrade waste and other complex matters [9]. The ability of bacteria to degrade Xenobiotics such as polythene has been ascribed to the secretion of certain enzymes [16]. These enzymes are primarily secreted as part of nutrition processes in the organisms [9]. Carbon is an essential nutrient in every life form, including bacteria [18]. The ability of bacteria to degrade polythene is hinged on their search for carbon nutrients [9,18]. Thus, the needed carbon along with other constituents of the decomposing matter are released at the mineralisation stage of biodegradation [9].

Studies have demonstrated the presence of hydrocarbonoclastic bacteria in every habitat [19]. Hydrocarbonoclastic bacteria are bacteria with the ability to degrade polythene into constituent minerals with no adverse effect on the environment [9,19]. *Pseudomonas* and *Micrococcus* spp. have been severally isolated and reported for LDPE degradation [9,16,13,20, 21]. Biodegradation of LPDE is said to follow the

colonisation at the surface and subsequent release of degrading substances by the microbes [13]. Proof of biodegradation is measured variously including increase in the cell count of the degraders [13].

Carbon is an essential nutrient required for the growth of virtually all life forms. In bacteria, growth follows the presence of the needed nutrient, with carbon again integral [22]. Growth in bacteria is however measured in terms of increase in their population due to their microscopic nature. Bacterial growth pattern follows the sigmoid curve and can be measured variously. Optical density offers an accurate method for measuring bacterial growth due to the automation and use of sensitive spectrophotometer [13,23]. The spectrophotometer measures the absorbance and is preferred as a cost effective and more specific method [23,24].

This study aimed at the isolation and characterisation of bacteria capable of degrading LDPE from farmlands and waste dump sites in Nsukka and monitoring their growth on LDPE as a sole carbon source. It was hypothesised that ability of the bacteria to grow on polythene as a carbon source shall prove its ability to degrade this important environmental pollutant.

2. MATERIALS AND METHODS

2.1 Sample Collection

Partially decomposed LDPE were collected within Nsukka metropolis in Enugu State at coordinates of 6.8429°N and 7.3733°E. Samples were taken from six (6) strategic locations (S1 to S6) consisting of farmlands and waste dumps. Samples were collected from a depth of 10 cm, wrapped in sterile polythene bags and transported to the Laboratory for storage at 4°C.

2.2 Enhanced Synthetic Medium

Mineral Salt Media (MSM) as described by Orr et al. [25] was used to study the ability of bacteria to grow on LDPE as their sole carbon source. Constituents of the MSM were 1.0 g NH₄NO₃, 0.1 g Yeast extract (LAB), 0.2 g MgSO₄·7H₂O, 1.0 g K₂HPO₄, 0.1 g CaCl₂·2H₂O, 0.15 g KCl. Also, 1.0 mg of each of the following microelements were added: FeSO₄·6H₂O, ZnSO₄·7H₂O, MnSO₄ and 10 g of pulverised LDPE. All constituents were dissolved in a conical flask containing 1 L of

distilled water and gently heated to ensure even mix.

2.3 Cultivation of Bacteria from Partially Degraded LDPE Samples

Sterile 10 ml MSM in test tubes were inoculated with 2 cm by 2.5 cm polythene sample after carefully dislodging them from sand. The inoculated test tubes were incubated at 37°C for 8 days. Prior to inoculation, the optical density (OD₆₀₀ nm) of the MSM in test tubes were recorded as control. All optical density (OD₆₀₀ nm) measurements were done with spectrophotometer (Spectronic 21D Milton Roy, USA).

Ability of isolates to use polythene as sole carbon source was studied as previously described [26]. Briefly, test tubes containing 3 ml of the enrichment medium were inoculated with a loop full of the cultivated culture. Tubes were incubated at temperatures between 23-25°C for 15 days. The ability of the organisms to grow on LDPE as sole carbon source was measured in terms of changes in optical density (OD₆₀₀ nm) at 24 h interval starting from day 4.

2.4 Isolation and Identification of Polythene Degrading Bacteria

The organisms were resuscitated by aseptically streaking a loop full of enrichment culture suspension on nutrient agar (NA) plates. All NA plates were then incubated at 37°C for 24 h to obtain pure culture. Discrete isolates were stabbed into bijou bottle slants and stored at 5°C until needed for identification.

Identification of isolated organisms involved microbiological and biochemical procedures as already described [27-30]. Biochemical tests performed include Sulphide, Indole, Motility, Catalase, Oxidase, Coagulase, Oxidation-Fermentation, Urease and Sugar fermentation tests.

Spores are formed by bacteria when subjected to stress such as depriving them of cheap carbon source. Further identification of isolates employed endospore staining [31].

2.5 Study of the Effect of Temperature on the Use of LDPE as a Carbon Source

The effect of 2 different temperatures (37°C and 50°C) on the growth of organisms on LDPE enriched MSM was studied. Ten millilitres of

MSM was dispensed into sterile test tubes and the optical density (OD₆₀₀ nm) taken as control. Then, organisms were introduced into normal saline to maintain osmotic pressure before a loop full was transferred into test tubes; 2 each for 37°C and 50°C respectively. All set up was incubated for 15 days and absorbance measured on daily basis after 48 h.

2.6 Statistical Analysis

All data were tested for significance using the paired t test using Minitab 15 English Ink. All testing were done at 95% confidence interval and significance stated if $p \leq 0.05$. Pearson's correlation coefficient was used to study the effect of time on degradation by the two organisms.

3. RESULTS AND DISCUSSION

Isolates from the 6 different sites were labelled IS₁, IS₂, IS₃, IS₄, IS₅ and IS₆ to denote sampling sites 1 to 6 (S1 to S6). Summary of results of the morphological and biochemical test is shown in Table 1. Efforts were made to isolate only *P. aeruginosa* and *Micrococcus* spp. for degradation of LDPE. Based on morphology and biochemical tests, *P. aeruginosa* was isolated from sites S1, S3 and S4 while *Micrococcus* sp. Were isolated from sites S2, S5 and S6.

All isolates were screened for their ability to grow on LDPE as a sole carbon source. This will demonstrate that they are able to degrade the ethylene polymer, polythene, to access the carbon for nourishment. Bacterial growth was measured in terms of optical density (OD₆₀₀ nm) as shown in Tables 2 and 3.

The effect of temperature on the rate of degradation of LDPE by the two isolates was also studied. Temperature is an important parameter, necessary for bacterial proliferation. Tables 2 and 3 show the growth of both *P. aeruginosa* and *Micrococcus* sp.

Fig. 1 combined growth of *P. aeruginosa* in LDPE at 37°C and 50°C while Fig. 2 shows the same information for *Micrococcus* sp.

The six distinct colonies isolated were identified to be *P. aeruginosa* and *Micrococcus* sp. Both organisms have been previously isolated and shown with enormous potentials for the degradation of LDPE [9,13,16,20,21]. The

current study is in agreement with the previous studies. These studies highlight *Pseudomonas* as a choice organism in the degradation of LDPE. However, the current study also noted enormous biodegradation potentials in *Micrococcus* sp.

Degradation of LDPE involves depolymerisation possible when organism possess exoenzymes and endoenzymes to drive the process [16,32-34]. Biodegradation involves three distinct phases; biodeterioration, depolymerisation and mineralisation [9]. It is in fact at the mineralisation (last) phase that the much-desired carbon is accessed by the organisms. The ability of organisms to grow on polythene as a sole carbon will impact on the growth of organisms in the enriched MSM used. Usually, the exoenzymes cleave the complex hydrophobic polymers into simpler hydrophilic monomers. LDPE are recalcitrant and tough to degrade due to their hydrophobicity [9]. Peroxidase is an important enzyme in the degradation of LDPE and has been demonstrated in *P. aeruginosa* and *Micrococcus* sp. [33,35]. These organisms depolymerise and make the polythene polymers more accessible to the endoenzymes that ensure mineralisation stage is reached. This mineralisation stage is typically the carbon nutrient availability stage. This stage is rewarding to the organisms and beneficial to the ecosystem. Thus, organisms in this process must drive the degradation to the end as that is the only assurance for reaching the desired nutrients.

Temperature was of essence on the degradation of LDPE. Both organisms showed significant difference in degradation rate at 50°C as compared to 37°C. This observation may be explained by various factors. The optimum growth temperature for *P. aeruginosa* is 37°C [36] and could explain the higher rate of degradation of LDPE at 37°C. However, the growth degradation at 50°C was also not dismal. Significant degradation was observed and could be explained by a wide temperature range for *Pseudomonas* sp. Some studies have reported growth temperature range of 5°C to 45°C for *Pseudomonas* [37]. Polythene degradation requires moderately high temperature for the oxidation [38]. Another study by Muhonja et al [9] reported improved LDPE degradation at 50°C. Similarly, *Micrococcus* sp. grows optimally at temperature ranging from 25°C to 37°C [39].

Table 1. Identification of *Pseudomonas aeruginosa* and *Micrococcus* sp. for LDPE degradation

Isolates tests	S1	S2	S3	S4	S5	S6
Morphology	Green rods	Cocci	Green rods	Green rods	Cocci	Cocci
Gram reaction	-	+	-	-	+	+
Motility test	+	-	+	+	-	-
Spore formation	-	-	-	-	-	-
Oxidase test	+	-	+	+	-	-
Catalase test	+	+	+	+	+	+
H ₂ S	-	-	-	-	-	-
Coagulase test	-	-	-	-	-	-
Indole test	-/+	-	-/+	-/+	-	-
O F Test	+	+	+	+	+	+
Urease test	-	Acid alone	-	-	Acid alone	Acid alone
Lactose test	Acid alone	Acid and Gas	Acid alone	Acid alone	Acid and Gas	Acid and Gas
Sucrose test	-	Acid alone	-	-	Acid alone	Acid alone
Glucose test	Acid and Gas	Acid and Gas	Acid and Gas	Acid and Gas	Acid and Gas	Acid and Gas
Mannitol test	Acid and Gas	-	Acid and Gas	Acid and Gas	-	-
Probable organism	<i>Pseudomonas aeruginosa</i>	<i>Micrococcus</i> sp.	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Micrococcus</i> sp.	<i>Micrococcus</i> sp.

This explains the significant difference observed in LDPE degradation, higher at 37°C as compared to 50°C. As expected, bacterial growth at 50°C will not be best, though degradation activities continued.

Table 2. Optical density of *P. aeruginosa* and *Micrococcus* sp. growing on LDPE at 37°C

Time (days)	<i>Pseudomonas aeruginosa</i>	<i>Micrococcus</i> sp.
Control	0.088	0.088
After inoculation	0.103	0.99
4	0.186	0.19
5	0.200	0.206
6	0.193	0.269
7	0.240	0.258
8	0.288	0.29
9	0.300	0.3
10	0.318	0.5
12	0.324	0.51

Time was important to the degradation process. It is a known factor in the bacterial growth. The growth of the degrading bacteria studied in LDPE showed a close correlation to time. This is evidenced by the high correlation coefficients obtained for both organisms. The bacterial growth curve otherwise known as the sigmoid curve, has distinct phases, describing the various growth stages [31]. The current study showed a trend similar to the sigmoid curve, but seemed limited by the incubation/study time. The growth in this study appeared to be in the exponential phase when the study ended. It is believed that if

given more time, degradation could have still continued at an exponential level. This further demonstrates the LDPE degradation potentials of the test organisms.

Table 3. Optical density of *P. aeruginosa* and *Micrococcus* sp. growing on LDPE at 50°C

Time (days)	<i>Pseudomonas aeruginosa</i>	<i>Micrococcus</i> sp.
Control	0.088	0.088
After inoculation	0.103	0.99
4	0.166	0.172
5	0.190	0.173
6	0.190	0.193
7	0.214	0.4
8	0.244	0.42
9	0.282	0.5
10	0.293	0.7

Overall, our study showed increase in optical density of the culture medium. This has been related to bacterial growth in this study. Bacterial growth is usually by binary fission leading to increase in number of colonies. This has led to increase in the cloudiness of culture media and thus increase in the OD₆₀₀. The finding of this study agree with previous studies [9]. The studies by Muhonja et al., [9] measured bacterial growth successfully by spectrophotometer. In the present study, both *P. aeruginosa* and *Micrococcus* sp. grew by the degradation of LDPE as a carbon source. This is confirmed by the increase in optical density in both organisms over time.

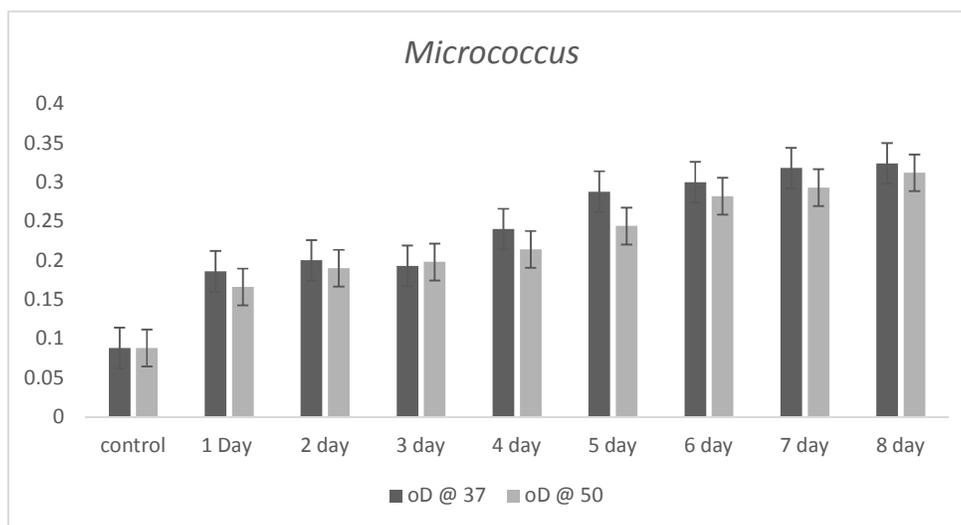


Fig. 1. Effects of temperature on micrococcus degradation of LDPE

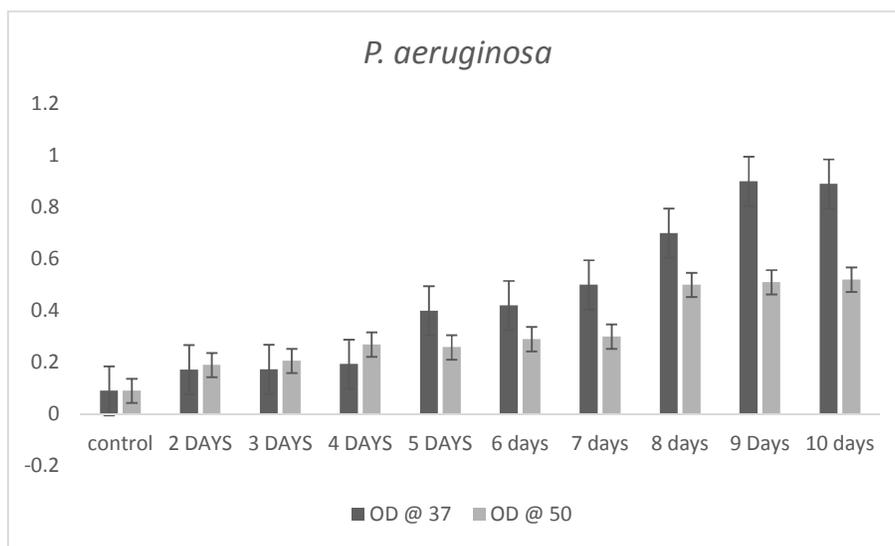


Fig. 2. Effects of temperature on *P. aeruginosa* degradation of LDPE

4. CONCLUSION

P. aeruginosa and *Micrococcus* spp. demonstrate high degradation potentials for LDPE. They offer great hope in environmental remediation for plastic pollution which has become a global environmental challenge. An understanding of other degrading factors could enhance the potentials already shown by these organisms. Adequate sorting and possible pretreatment of polythene waste could enhance the ease of degradation of LDPE and restore the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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