Biosurfactants as facilitators in Biodegradation of Low-Density Polyethylene (LDPE)

C. F. Nnaji1*, E. C. Ogu1 and O. B. Akpor2

1Department of Chemical Engineering, Landmark University, Omu-Aran, Kwara State, Nigeria.
2Department of Microbiology, Landmark University, Omu-Aran, Kwara State, Nigeria.
*Corresponding author: nnaji.chioma@lmu.edu.ng

Abstract

Low density polythene (LDPE) is amongst the highest produced synthetic plastic and also largely plagued with ineffective disposal management. Strategies to remedy its ineffective disposal have been underway and at the forefront is biodegradation due its positive environmental impact. This study reports on the preliminary investigation into surface chemistry using biosurfactants as facilitators for the biodegradation process of LDPE. Synthesized biosurfactants from isolated soil microbes, Bacillus subtilis and Pseudomonas aeruginosa were used in the biodegradation study along with pure cultures of the organisms themselves. 0.84mg/ml was the highest amount of biosurfactant synthesized under static conditions at 25°C. Supplementing with biosurfactants increased the biodegradation efficiency by at least 1.2 % compared to using the microbes alone during a 30 d incubation period. Percentage weight loss of LDPE bags was used as a measure of biodegradation in this study and 3.3% weight loss was the highest observed for a single organism when augmented with biosurfactants compared to 1.9% when used alone.

Keywords: Biosurfactants, LDPE, Biodegradation, Pseudomonas aeruginosa, Bacillus subtilis, Yeast and bacterial strains.

1. Introduction

Plastics are ubiquitous commodities that affect virtually all areas of life from personal hygiene, packaging, food preservation, electronics, housing to automobile [1]. As functionally relevant as plastics are, they are considered a global pollutant posing threat to the environment, ecological system, fauna and public health due to their non-degradable property causing them to linger for centuries in oceans and soils and poor disposal management [2]. A persistent group of synthetic plastics in dumpsites in most developing countries belong to
low-density polyethylene (LDPE), which is mostly utilized in portal drinking water packaging and has a global estimation of 500 billion plastic bags annual usage [3-5].

LDPE are a group of polymers consisting of long-chain ethylene bonds with high branching network, which contributes to its less compact crystalline structure with amorphous regions. This lends to its characterization as a low-density, highly flexible material and therefore its use in flexible film and a variety of packaging applications [6]. It is thus classified as a semi-crystalline, soft & flexible and versatile material. Its crystallinity - tightly packed molecular nature, along with high molecular weight and carbon backbone chains affects many of its characteristics most importantly, its degradation. This renders them hydrophobic and non-biodegradable persisting for decades in the environment [7]. Some strategies to remediate LDPE pollution include production of biodegradable plastics [8] and recycling, which is mostly mechanical albeit problematic as LDPE films gets tangled and perturbs the process and thus, still in need of redressing [9]. As a result, lots of research has been directed towards biodegradation of LDPE.

In the biodegradation of LDPE, several microbes have shown evidence of degradation with *Pseudomonas* sp. [10-12] and *Bacillus* sp. [13] as prominent bacterial degraders. Using *Pseudomonas* sp. 20.54% degradation of polythene was achieved in 30 days using polythene as sole carbon source [11] and 0.95% in 45 days [12]. 1.5% degradation after 60 days incubation using LDPE as sole carbon source was reported for *Bacillus* sp. [13] while Harshvardhan et al reported 1.5 – 1.75% in 30 days using the same microbe [14].

Due to the recalcitrant nature of LDPE, certain pretreatments have been used to increase the rate of degradation; pre-treatment with nitric acid resulted in 50.5% LDPE degradation in 60 days using *P. aeruginosa* months [15], Vimala observed that 72 h UV irradiation pre-treatment of LDPE film grown in *B. subtilis* and supplement with biosurfactant yielded 9.26% degradation in 30 days [16].

Surfactants are amphipathic compounds that mediate the immiscibility between polar and nonpolar substances by reducing their surface tension. They enable the interaction of such substances by possessing both hydrophobic ends that could be fatty acids, unsaturated or saturated hydrocarbon chains and hydrophilic ends either peptide ions, acids, alcohol, phosphates, or saccharides [17]. Surfactants effect surface changes beneficial for the onset of
degradation of hydrophobic polymers such as LDPE and been chemically produced may have deleterious impacts on the environment [18-19]. Hence, the use of Biosurfactants – surfactants synthesized from a plethora of microbes including *Pseudomonas sp.* [20], *Bacillus sp.* [21] and soil isolates in this study to accelerate the biodegradation of LDPE.

2. Methodology

**Collection of samples and microbial isolation**

Soil sampled from Landmark University Teaching and Research Farms were analysed for microbial isolation, using standard procedures. A yeast and two bacteria strains yet to be identified isolated from the soil were probed for biosurfactant production while *Pseudomonas aeruginosa* and *Bacillus subtilis* (known biosurfactant producers) were gotten from the Department of Microbiology, Landmark University, Nigeria. Pure cultures of the strains from the soil sample were obtained by re-streaking them on nutrient agar and sabourand dextrose agar plates at 30 °C for 24 h, which were transferred to agar slants and stored at 4 °C.

**Optimization of culture conditions for biosurfactant production**

The growth media were according to the following composition per L: glucose, peptone and sodium chloride 5 g and Yeast extract 3 g. 250 mL of the media was prepared into fifteen 500 mL conical flasks and sterilized at 121°C for 15 mins. Subsequently, 1 ml o/n cultures of the test microbes (*P. aeruginosa*, *B. subtilis* and soil strains) were inoculated into the sterilized media with the experimental setup optimized using two temperatures (25°C and 37°C) with an optional agitation for 5 d. The growth rates were monitored daily at an optical density of 600nm while biosurfactant production were measured using the tests described below. All tests were done in triplicates. Growth rates were estimated according to the equation:

\[
\text{Growth rate (d}^{-1}) = \frac{\ln(C_1) - \ln(C_0)}{t_1 - t_0}
\]

With initial and final absorbance reading as \(C_0\) and \(C_1\) respectively at corresponding times \(t_0\) and \(t_1\).
Screening for Biosurfactant activity

Evidence for the production of biosurfactant by the organisms in this study was tested in terms of their ability to form emulsions and quantified using emulsification index.

Emulsification index

The ability to form emulsion was determined by mixing equal volumes (2 ml) of culture broth devoid of cells and Sesame oil in test-tubes while vortexing for about 2 min. The mixtures were set aside at room temperature for 24 h and height of the resulting emulsion were recorded and used for the calculation of the emulsification index at 24 h ($E_{24}$), which is a measure of the stability of emulsions formed according to the equation

$$
\% E_{24} = \frac{\text{Height of emulsion formed}}{\text{Total height of solution}} \times 100
$$

Extraction and Quantification of Biosurfactant

After 96 h growth at the various culture conditions, the culture broth was centrifuged at 10,000 rpm for 10 min to remove the cells debris while the supernatant containing the synthesized biosurfactant was further subjected to extraction. The supernatant was acidified to pH 2.0 by the addition of 6N hydrochloric acid and left overnight at 4°C for precipitation. Thereafter, the solution was centrifuged at 10,000 rpm for 5 min to recover the pellets to which chloroform and methanol were added in the ratio 2:1. The organic phase was recovered using a separating funnel, concentrated by freeze-drying and resulting sediments weights noted.

Oil displacement Test

The activity of extracted biosurfactant was verified by testing its ability to reduce interfacial tension between polar and non-polar solvents. To do this, 40ml of distilled water was placed in an empty crucible of diameter 8cm to which 20μl Sesame oil was added forming a thin oil layer. 10μl of biosurfactant was pipetted to the centre of the solvents and the diameter of clear water zone, if any, was recorded; 10μl distilled water was used as control. The oil displacement area (ODA) was calculated thus:

$$
\text{ODA} = \frac{22}{7} \times (\text{radius})^2
$$
Application in Biodegradation of LDPE

For the biodegradation of LDPE, the media was used was same as that for the biosurfactant synthesis, except that LDPE would be used as the sole carbon source in place of glucose. LDPE bags were cut into 2x2 cm strips, initial weight noted, sterilized by immersing in 70% ethanol for 30 mins, rinsing in distilled water and finally dried at 40°C in an incubator. The experiment was set up as follows: 15 ml o/n cultures of the 5 organisms were separately inoculated into 250ml conical flask containing 150ml sterile media to which 6 sterilized LDPE strips were added along with 0.4 mg/ml biosurfactant synthesised from that particular organism. To maximise biosurfactant synthesis from the organism in the conical flask, the optimized conditions of static 25°C incubation were used and the experiment lasted 30 days. The control setup were microbes and LDPE strips without biosurfactant supplementation and LDPE strips in culture media with neither microbes nor biosurfactant. The experimental procedure was performed in triplicates. Sampling was done at 5 d interval, whereby a strip of LDPE was aseptically withdrawn from each flask, washed with 2% sodium dodecyl sulfate (SDS) for 3 h, thoroughly rinsed in distilled water, dried in an incubator at 40°C. The percentage weight loss was used as a measure for biodegradation according to the equation

\[
\text{% Weight Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100
\]

Results and Discussion

Biosurfactant production by microbes

Emulsions result from the dispersion of a liquid phase as droplets into another liquid phase – in solutions that are otherwise not homogeneous with each other. Biosurfactants bring about such emulsion formation by reducing the interfacial surface tension between the immiscible solvents and the emulsion formation ability in 24 h is measured by the emulsification index \((E_{24})\) [22]. \(E_{24}\) varies between microbes, growth media and the type of hydrophobic solvent used. Furthermore, the height of emulsion formed is indicative of the amount of biosurfactant present in the cell-free culture broth and therefore its surface reducing ability. This test was used as the initial screening for the synthesis of biosurfactant.
The emulsification observed was dependent on the laboratory conditions used. At culture conditions 37 °C with and without agitation, growth was detected in all organisms but emulsions were not. Generally, agitating the culture flasks did not seem to favour emulsification, as even at 25 °C which favoured both growth and emulsification, emulsions were not observed in Strain 2 when agitated as shown in Table 1. This agrees with Pereira et al, who reported biosurfactant production by 3 Bacillus subtilis strains grown at 40 °C without agitation in various carbon and nitrogen sources [23]. Additional reports also show increase biosurfactant production in less aerated environments; agitation promotes aeration to the cells [24-26]. These results are shown in Fig. 1 and thus, prompted the use of these conditions for subsequent experiments.

Table 1: Biosurfactant detection via emulsion formation at different incubation periods and agitation for the test organisms.

| Organism          | Incubation Period (h) | 25 °C without agitation | 25 °C with agitation | 37 °C with & without agitation |
|-------------------|-----------------------|-------------------------|----------------------|-------------------------------|
| Strain 1          | 24                    | +VE                     | +VE                  | -VE                           |
|                   | 48                    | +VE                     | +VE                  | -VE                           |
|                   | 72                    | +VE                     | +VE                  | -VE                           |
|                   | 96                    | +VE                     | +VE                  | -VE                           |
| Strain 2          | 24                    | +VE                     | -VE                  | -VE                           |
|                   | 48                    | +VE                     | -VE                  | -VE                           |
|                   | 72                    | +VE                     | -VE                  | -VE                           |
|                   | 96                    | +VE                     | -VE                  | -VE                           |
| Strain 3          | 24                    | +VE                     | +VE                  | -VE                           |
|                   | 48                    | +VE                     | +VE                  | -VE                           |
|                   | 72                    | +VE                     | +VE                  | -VE                           |
|                   | 96                    | +VE                     | +VE                  | -VE                           |
| Bacillus subtilis | 24                    | +VE                     | +VE                  | -VE                           |
|                   | 48                    | +VE                     | +VE                  | -VE                           |
|                   | 72                    | +VE                     | +VE                  | -VE                           |
|                   | 96                    | +VE                     | +VE                  | -VE                           |
| Pseudomonas Aeruginosa | 24            | +VE                     | +VE                  | -VE                           |
|                   | 48                    | +VE                     | +VE                  | -VE                           |
|                   | 72                    | +VE                     | +VE                  | -VE                           |
|                   | 96                    | +VE                     | +VE                  | -VE                           |

+VE and -VE denoting emulsion formed and emulsion not formed, respectively
Figure 1: Effect of culture conditions on emulsification index ($E_{24}$) with (A) as positive emulsion formation and (B) as negative. The heights of the emulsions are evident in A with oil as top layer and bottom layer cell-free culture.

Glucose has been shown to be a good carbon source for biosurfactant production in both *P. aeruginosa* and *Bacillus sp.* [27] in terms of $E_{24}$, in the region of 27 – 35% for. In this study, Strain 1 had the highest $E_{24}$ and strain 2 the least with 38.6 and 32.2% respectively. The results are enumerated in Table 2. Values obtained for *Bacillus subtilis* were similar to those in [23]. These results also paralleled those of oil displacement area test, the maximum observed was 41.86 cm$^2$ for strain 1 and the least was Strain 2 with 30.2 cm$^2$. El-Sheshtawy, work on *P. Aeruginosa* biosurfactant production and antimicrobial, he also observed *P. Aeruginosa* to give an oil area displacement of 38.5 [28].

The oil displacement test is predictive measure for surfactant’s ability to reduce interfacial surface tension, which precedes the formation of an emulsion and thus increases access to the hydrophobic substance. Similar to $E_{24}$, it varies with the substances, organisms and process conditions. The clear area created by dispersion indicates high activity of the biosurfactant. Biosurfactant activities and amount synthesized by the microbes are shown in Table 2.
0.84 mg/ml was largest amount of biosurfactant recovered with 0.4 as the least recovered, again for strains 1 and 2 respectively. It is heavily suspected that strain 2 is a yeast based on the results obtained from streaking the strains on sabourand dextrose agar plates (not shown). Thus, cultivating in a media that supports the growth of yeast may have increased its yield of biosurfactant as there is evidence of biosurfactant synthesis from yeast [29]. Lastly, a linear correlation between the incubation time and the amount of biosurfactant synthesized as well as their resulting biosurfactant activities (E24 and oil displacement area) seemed to exist.

Table 2: Summary of Biosurfactant activities in terms of emulsification index and oil displacement area and amount synthesized for the test organisms statically incubated at 25 °C.

| Samples      | Emulsification Index (%) | Oil Displacement Area (cm²) | Amount of Biosurfactant synthesized (mg/ml) |
|--------------|--------------------------|-----------------------------|------------------------------------------|
| Strain 1     | 38.6                     | 41.86                       | 0.84                                     |
| Strain 2     | 32.2                     | 30.2                        | 0.4                                      |
| Strain 3     | 35                       | 38.5                        | 0.77                                     |
| B. Subtilis  | 32.8                     | 33.2                        | 0.53                                     |
| P. Aeruginosa| 33.33                    | 38.5                        | 0.75                                     |

**Biodegradation of LDPE**

Biodegradation of plastics generally involves fragmentation of the polymer into monomers, which are then assimilated by microbial cells and utilized during metabolism to either carbon dioxide and water (aerobic process) or methane and carbon dioxide (anaerobic process) [30]. The fragmentation process is plagued by the hydrophilic nature of most microbial cell surfaces to that of the hydrophobic polymer. This impairs the microbe’s ability to access and attach onto the hydrophobic polymer surface but can be improved by biosurfactants, which reduce the said surface tension allowing for polymer solubility thereby increasing polymer surface area and promoting microbial attachment to polymer surface [31-32].

In all test organisms, supplementing with biosurfactants resulted in higher degradation of LDPE compared to using microbes alone. At least, biodegradation efficiency was increased
by 1.2% across all organisms except for Strain 2, where there was no significant weight loss in the LDPE strip. Strain 1 had the highest % weight loss both when microbes were used alone and when augmented with biosurfactants (1.9% & 3.3% respectively) while *B. subtilis* had the least with 1.4% when only microbes were used and 2.6% when biosurfactants were added but had the highest fold increase of 1.86. These results are comparable to [14] where 1.75 ± 0.06% weight loss of LDPE film was reported using *B. subtilis. % biodegradation results are shown in Fig 2.

There was no noticeable weight change in the control experiment containing neither microbes nor biosurfactant rather, a linear correspondence between % weight loss of LDPE and incubation time was observed for the experiments during the 5 d periodic sampling.

![Figure 2: Gravimetric determination of LDPE bags biodegraded during 30-d static incubation at 25 °C superimposed with amount of Biosurfactant synthesized by test organisms under similar conditions in a different experiment.](image)

Going by the previous results of the biosurfactant production by the organisms, one can assume that the organisms will synthesize new biosurfactants by almost the same margin as before and hence the biodegradation study would follow the same trend as that of biosurfactant production.
Ultimately identifying the type of biosurfactant produced by each organism would elucidate surface activities in their degradation of LDPE films and inform on strategies for improvement and therefore more yield.

Conclusion

Emulsification index and oil displacement tests confirmed the synthesis of biosurfactant by both yeast and bacterial cells used in the study. Static incubation at 25°C was conducive for the production of biosurfactant rather than agitation. The biodegradation of LDPE, a semi-crystalline hydrophobic polymer was bolstered by the surface chemistry modifications by augmenting with biosurfactants in addition to the use of microbes that also synthesize them.

Recommendation

Conducting more surface chemistry investigations to better understand the mechanism by which biosurfactants affects surface changes in the fragmentation of synthetic plastics would be beneficial to this research area as would a complete biodegradation of the polymers to mineralization. This can help actualize the chemical recycling of LDPE.

Acknowledgement

The authors acknowledge the financial support offered by Landmark University in the actualization of this research work for publication.

References

1. Freinkel Susan. (2011), “Plastic, A toxic Love Story”, Houghton Mifflin Harcourt Publishing Company, New York, USA.
2. Jafari, A. et al. (2015) ‘Biological synthesis of silver nanoparticles and evaluation of antibacterial and antifungal properties of silver and copper nanoparticles’, *Turkish Journal of Biology*, 39(4), pp. 556–561. doi: 10.3906/biy-1406-81.
3. PlasticsEurope (2015) Plastics—The Facts 2015. An analysis of European plastics production, demand and waste data. Available at: http://www.plasticseurope.org/Document/plastics—the-facts-2015.aspx.
4. Roy PK, Surekha P, Tulsi E, Deshmukh C, Rajagopal C. (2008), Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. Polym. Degrad. Stab. 93:1917–1922. doi: 10.1016/j.polymdegradstab.2008.07.016.
5. Ragaert, K.; Delva, L.; Van Geem, K. Mechanical and chemical recycling of solid plastic waste. *Waste Manag.* **2017**, 69, 24–58.
6. Wilkes, R. A. & Aristilde, L. (2017), ‘Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges’, Journal of Applied Microbiology 123, 582-593
7. Burd, D. (2008), ‘Plastic Not Fantastic. In, Canada. http://wwsef.uwaterloo.ca/archives/2008/08BurdReport.pdf.
8. Nnaji, C. F. et al (2019), ‘Biopolymer Synthesis and Detection by Soil Bacteria and Yeast’ Journal of Physics: Conference Series 1378(2019) 042044 doi:10.1088/1742-6596/1378/4/042044.
9. Newman, J. P. (2014), ‘Container Nursery Production and Business Management Manual’ UCANR Publications, California
10. Kyaw, B. M., et al (2012). Biodegradation of Low-Density Polythene (LDPE) by Pseudomonas Species. Indian journal of microbiology, 52(3), 411–419. https://doi.org/10.1007/s12088-012-0250-6
11. Kathiresan, K., (2003), "Polythene and plastics-degrading microbes from the mangrove soil", Rev. Biol. Trop., 51(3): 629–633.
12. Tribedi, P. and Sil, A.K. (2013c), “Low-density polyethylene degradation by Pseudomonas sp. AKS2 biofilm”. Env. Sci Pollut. Res 20, 4146–4153.
13. Gupta, K. K. & Devi, D. (2019), ‘Biodegradation of low-density polyethylene by Selected Bacillus sp. J Sci, 32(3): 802-813;
14. Harshvardhan, K.; Jha, B. (2013), ‘Biodegradation of low-density polyethylene by marine bacteria from pelagic waters Arabian Sea’, India. Mar. Pollut. Bull. 77, 100–106.
15. Rajandas, H., et al (2012) A novel FTIR-ATR spectroscopy-based technique for the estimation of low- density polyethylene biodegradation. Polym Test 31, 1094–1099.
16. Vimala, P. P. & Mathew, L. (2016), ‘Biodegradation of Polyethylene using Bacillus subtilis’, Procedia Technology 24 pp. 232-239
17. W. Katemai, et al. (2008), ‘Purification and characterization of a biosurfactant produced by Issatchenkia orientalis SR4’ J. Gen. Appl. Microbiol., 54, pp. 79-82
18. Rosenberg, E. & Ron, E.Z. (1999), ‘High- and low-molecular-mass microbial surfactants’, Applied Microbiology and Biotechnology Volume 52, Issue 2, pp. 154-162
19. Desai, J.D. & Banat, I.M., (1997), ‘Microbial production of surfactants and their commercial potential’, Microbiology and Molecular Biology Reviews Volume 61, Issue 1, pp. 47-64
20. Nanda, S.; Sahu, S.S. Biodegradability of polyethylene by Brevibacillus, Pseudomonas, and Rhodococcus spp. N. Y. Sci. J. 2010, 3, 95–98.
21. Das, M.P.; Kumar, S. An approach to low-density polyethylene biodegradation by Bacillus amylolytiquefaciens. 3 Biotech 2015, 5, 81–86.
22. Amodu, O. S., Ntwampe, S. K., and Ojumu, T. V. (2014). "Emulsification of hydrocarbons by biosurfactant: Exclusive use of agrowaste," BioRes. 9(2), 3508-3525.
23. Pereira, J. F. B. (2013). ‘Optimization and characterization of biosurfactant production by Bacillus subtilis isolates towards microbial enhanced oil recovery applications’ Fuel 111 259–268.
24. Kim, S.H., et al (1997), ‘Production and properties of a lipopeptide biosurfactant from Bacillus subtilis C9’, J. Ferment. Bioeng. 84: 41-46.
25. Lin, S.C., et al (1994) ‘Continuous production of the lipopeptide biosurfactant of Bacillus licheniformis JF-2’, Appl. Microbiol. Biotechnol. 41: 281-285.
26. Ghribi D & Ellouze-Chaabouni S. (2011), ‘Enhancement of Bacillus subtilis lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration’. Biotechnol. Res. Int:1-6. http://dx.doi.org/10.4061/2011/653654.
27. Suwansukho, et al. (2008), ‘Production and applications of biosurfactant from Bacillus subtilis MUV4’ Songklanakarin J. Sci. Technol. 30, 87-93.
28. El-Sheshtawy, H. S & Doheim, M. M. (2014), ‘Selection of Pseudomonas aeruginosa for biosurfactant production and studies of its antimicrobial activity’, *Egyptian Journal of Petroleum* 23, 1-6.

29. Gautam G, et al. (2014), ‘A Cost-Effective Strategy for Production of Bio-surfactant from Locally Isolated Penicillium chrysogenum SNP5 and Its Applications’. J Bioprocess Biotech 4:177 doi: 10.4172/2155-9821.1000177.

30. Kolvenbach, B.A. et al (2014), ‘Emerging chemicals and the evolution of biodegradation capacities and pathways in bacteria’. Curr Opin Biotechnol 27, 8–14.

31. Chang, J. S. et al (2004), ‘Enhancement of phenanthrene solubilization and biodegradation by trehalose lipid biosurfactants’. Environ Toxicol. Chem. 23, 2816–2822.

32. Santos, D. K., et al (2016), ‘Biosurfactants: Multifunctional Biomolecules of the 21st Century’. Int J Mol Sci 17, 401, doi: 10.3390/ijms17030401.