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Research Article

Screening of Fungal Isolates for Biodegradation Potentials of Low-Density Polyethylene from Selected Dumpsites

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ABSTRACT

Polyethylene is the most commonly used synthetic plastic and poorly degraded in natural environments. This study was designed to evaluate biodegradation potentials of low-density polyethylene by fugal isolates from Station Market, Kaduna State, Nigeria. Soil samples were collected at a depth of 20cm using a sterilized hand towel and taken to microbiology laboratory, Kaduna State University. Fungi were isolated and each colony was repeatedly sub-cultured on freshly prepared SDA to obtain pure isolates. Pure separated single colonies were maintained on sterile slants at 4˚C for further investigation. Low-density polyethylene (LDPE) sheets were cut into bits and immersed in xylene and boiled for 15 minutes to dissolve and crushed. The LDPE powder was added to a mineral salt medium (MSM) containing salts in 1liter of distilled water after which the fungal isolates were introduced. Scanning electron microscopy and Fourier- Transform Infrared Spectroscopy (FTIR) analysis were used to determine the changes in the polymer bond of LDPE sheet after the biodegradation. The findings shows that *Aspergillus niger*, *Aspergillus flavus*, Brown rot and White rot fungi showed 21.1%, 16.1%, 17.6%, and 18.3% reductions in the polyethylene discs after incubation respective. Therefore, the study concludes that fungal species play a significant role in the degradation of LDPE and can be used in future studies for the degradation of complex plastic materials. Finally, the study recommends that *Aspergillus niger* and *Aspergillus falvus* be considered as potential candidates to degrade LDPE.

Keywords: Fingi; Screening; Biodegradation; Polyethyelene; Dumpsite

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INTRODUCTION

Synthetic and bio-based plastics technology was developed not only as expected to continue its unique properties for more and more innovative applications. The recalcitrance of some plastic items to biodegradation has led to an increase public concern about the hazards associated with plastic residues in the environment. Over the past ten years, this has led to efforts by scientific, industrial, and regulatory organizations to evaluate the exposure to and impacts of these macromolecular substances in various environmental compartments. Synthetic polymers have greatly benefited society and are unquestionably essential to modern living. Their low cost, ease of disposal, physical, chemical, and thermomechanical qualities, as well as their chemical and physical stability, make them highly advantageous materials for use in industrial processes and daily life. Around 300 million tons of synthetic polymers are generated annually in various parts of the world, and a significant portion of these polymers are released into the environment as industrial waste products (Paço *et al*., 2017).

Long chains of the thermoplastic polymer polyethylene (PE) are created by joining the component monomer ethylene. When ethylene enters a polymer, it truly transforms into ethane, and the straight segments of the polymers have the same structure as simple chain hydrocarbons. Low-Density Polyethylene, or LDPE, is the most significant polyethylene grade. The density range of 0.910-0.940 g/cm3 characterizes LDPE. At room temperature, it is not reactive unless powerful oxidizing chemicals are applied. It can tolerate constant temperatures of 80°C and brief excursions to 95°C. It comes in opaque or translucent varieties, is strong and flexible but brittle. More branching occurs in LDPE (on around 2% of the carbon atoms). Its resilience is higher, its tensile strength is lower, and its intermolecular forces are weaker. Its side branches result in a reduced density and less crystalline, more loosely packed molecules (Baculi *et al.,* 2017).

The ability of fungus and Streptomyces strains to attack degradable polyethylene, which is made up of discarded polyethylene bags containing 6% starch, was examined by Awasthi et al. (2017). Since all of the prior publications only showed activity after a minimum of 3–4 months, there is still more work to be done to isolate the proper kind of microbial strain that may promote breakdown of LDPE in a shorter amount of time. Biodegradability has been tested using a variety of analytical techniques, such as clear zone development, molar mass changes, weight loss measurement, and visual inspection. One of the main metabolic end products produced by aerobic microbes is carbon dioxide, which is created when carbon is oxidized using oxygen.

The study aims at evaluating and screening of fungi biodegradation potentials of low-density polyethylene from dumpsites.

MATERIAL AND METHODS

Study Area

The soil samples were collected from Station Market, Kakuri located in Kaduna South Local Government Kaduna, Nigeria. This site has large amounts of plastic wastes buried for a long time under natural conditions.

Sample Collection

Fifteen soil samples were collected at a depth of 20cm using a sterilized hand towel at three different locations and five samples were collected from each of the three locations (Abd El-Rahman *et al.,* 2020). Following this, the sample were transported to Microbiology Laboratory of Kaduna State University in a sterilized polyethylene bag and stored at 4^0C until required. The *polyethylene* (LDPE) used for this study was purchased from Cybex Laboratory, Kaduna North, Kaduna State, Nigeria.

Isolation, Cultural and Morphological Characteristics of Fungal isolates

One gram of each of the soil sample was aseptically weighed and transferred into a test tube containing 10mL of distilled water and allowed to stand for 30 minutes. One millilitre of the stock suspension was serially diluted and 1mL of dilutions 10^{-2} and 10^{-3} of each sample was placed on Sabouraud Dextrose Agar (SDA) plates into which 0.05 µg/ml chloramphenicol was added to inhibit bacterial growth (Sangale *et al.,* 2019). The plates were spread using sterile spreader and incubated at 25°C for a period of 5 days during, which they were carefully monitored and examined for growth (Rajeswari *et al.,* 2017). Each colony was continously sub-cultured on freshly prepared SDA to have pure isolates. Pure segregated single colonies were maintained on sterile slants at 4˚C for subsequent investigation (Rajeswari *et al.,* 2017).

Identification of Fungal isolates

Fungi were isolated using their cultural and morphological characteristics. The presumptive fungal isolates were macroscopically identified by carefully observing the colour of the colonies, colour of the reverse sides, texture as well as their growth patterns. Besides, the vegetative and reproductive structures of the fungi was viewed under compound light microscope using the Lacto-phenol cotton blue (LCB) staining technique. A drop of the lacto-phenol cotton blue stain was placed on clean microscopic slides and 0.5mm of the hyphae was aseptically picked using an inoculating needle. It was gently streaked on to the stain, then covered with a grease free, clean cover slip and observed under x40 objective lens. The characteristics of the fungal isolates was compared with the characteristics of known taxa using the scheme of Cheesbrough (2006).

Screening of Polyethylene Degrading Fungi

Preparation of LDPE Powder

After buying and cutting some LDPE sheets from the market, the sheets were submerged in xylene and

cooked for fifteen minutes. The residue and the dissolved LDPE film were then crushed with gloves on while still warm. The resulting LDPE powder was cleaned with ethanol to get rid of any remaining xylene. Overnight, the powder was dried at 60^0C in a hot air oven.

The following salts were added to 1 litre of distilled water to create Mineral Salt Medium (MSM) before LDPE powder was added: 1 g of K_2HPO_4; 0.2 g of KH_2PO_4; 1 g of NaCl; 0.002 g of $CaCl_2.2H_2O$; 0.005 gm of boric acid; 1 g of $(NH_4)2SO$; 0.5 g of $MgSO_4.7H_2O$; 0.001 g of $CuSO_4.5H_2O$; 0.001 g of $ZnSO_4.7H_2O$; 0.001 g of $MnSO_4.H_2O$; 0.01 g of $FeSO_4.7H_2O$.

Inoculation of fungal isolates into LDPE mixture

Fungal isolates from pure cultured samples were inoculated in the mixture above and incubated for 14 days under room temperature. After the 14 days, the absorbance of each sample was read. Fungal isolates which gave highest absorbance were further subjected to confirmation of their molecular characterization as described below (Sindujaa *et al.,* 2011).

Confirmation of Fungal Isolates

The DNA extraction, PCR Amplification and Sequencing Analysis of fungal cells were carried out based on the procedure described by Kumari *et al.* (2011).

Biodegradation of LDPE by Fungal Isolates

Seventy milligram of LDPE sheets was cut into small pieces of similar weight, disinfected with 70% of ethanol for 30 minutes and transferred to sterile water for 20 minutes. Four LDPE sheets of similar weight was placed in 100ml beaker containing mineral salt medium. To this, fungal cells was added and then incubated for 30 days at room temperature in a stationary position

Detection of Biodegradation of Polyethylene by Determination of the Weight Reduction

The treated LDPE films was recovered from the degradation medium and washed with 2 % (v/v) sodium dodecyl sulfate (SDS) solution and further rinsed with distilled water (Verma and Gupta, 2019). The washed LDPE film was air dried at room temperature before weighing and the percentage of weight loss determined (Abd El-Rahman *et al.,* 2020). The weight difference between initial and final weight indicates the extent of polythene utilization by the fungi. Percentage weight loss was determined using the formula:

Weight loss (%) = {(Initial Weight - Final Weight)/Initial Weight} x 100

Scanning Electron Microscopy (SEM)

Using a scanning electron microscope, the LDPE samples were magnified 500x, 1000x, and 1500x to examine the surface morphology both before and after biodegradation. An unquestionable and direct proof of fungal activity on the LDPE film was supplied by scanning electron micrographs taken of the material both before and after the treatment. The film's surface looked flawlessly smooth and clean prior to the treatment. Post-treatment micrographs, however, demonstrate varying degrees of fungal growth on the films where mycelia and conidia were physically visible attached to the surface. The topography photos of the treated films revealed pits, grooves, corrosion, and other features. These photos also indicated the attachment and growth of *Aspergillus flavus* and *Alternaria alternate*.

Fourier- Transform Infrared Spectroscopy

Using Fourier-Transform Infrared Spectroscopy (FTIR) analysis, the alterations in the polymer link of the LDPE sheet following biodegradation were ascertained. FT-IR (Fourier transformed infrared) 8400 S Schamdzu spectroscopy was employed to characterize the materials under analysis. Samples were homogenized using mortar agate and weighted in at 0.01 g using 0.01 g of KBr anhydrous. Transparent pellets were obtained by pressing the mixes using Graseby Specac vacuum hydraulic at 1.2 psi. The scanned sample traveled through the infrared spectrum, where it was detected and its spectrum was characterized by a detector that was connected to a computer. Typically, samples were examined within the 600–4000 cm-1 absorption region. The analysis's findings included the examined substances' chemical makeup, molecular binding form, and specific functional groups as

RESULTS

Fungal Isolates Obtained from soil samples collected from dumpsites in Kakuri Market

A total of 15 isolates were derived from soil collected from three (3) different location within the market dumpsites and were recorded as A, B and C.

for each location, five (5) samples were collected and inoculated, the number of distinct fungal colonies were recorded.

Screening of Fungal Isolates for their Ability to Degrade LDPE

A total of 38 isolates were subjected to screening among which four of the isolates shows appreciable utilization of LDPE by the fungal isolates and 2 of the isolates A14 and C41 which shows the highest utilization absorbance were further identified.

Weight Loss Estimation

In the biodegradation experiment, *Aspergillus niger, Aspergillus flavus*, and brown rot and white rot fungi showed 21.1%, 16.1%, 17.6%, and 18.3% percentage weight reduction of the polyethylene after incubation with the respective isolate for 30 days. Control showed no reduction in weight loss. The initial weight of LPDE was 17.0 mg before treated with *Aspergillus niger*, after treatment and incubation for 30 days in a shaking incubator, the final weight of plastic 13.4 mg was observed to have a significant weight loss of 4 mg and biodegradation of LDPE up to 21.1%, as shown in Table 4.7.

Table 1. Fungal Isolates obtained from Soil Samples Collected from Dumpsites in Kakuri Market

Sample Location	Sample Code	Number of Distinct Fungal Colonies
A	A1	4
	A2	2
	A3	4
	A4	2
	A5	3
B	B1	3
	B2	2
	B3	1
	B4	3
	B5	2
C	C1	2
	C2	2
	C3	4
	C4	3
	C5	1
Total		38

Keys: A = Sample Location 1, B = Sample Location 2, C = Sample Location 3

Table 2. Screening of fungal isolates for their ability to degrade LDPE

Sample Location	Isolate Code	Ability to degrade LDPE
A	A11	-
	A12	-
	A13	+
	A14	+++
	A21	+
	A22	+
	A31	+
	A32	-
	A33	-
	A34	-
	A41	-
	A42	-
	A51	-
	A52	-
	A53	++
B	B11	-
	B12	-
	B13	-
	B14	-
	B21	-
	B22	-
	B31	-
	B41	-
	B42	-
	B43	-
	B51	-
	B52	-
C	C11	-
	C12	+
	C21	+
	C22	-
	C31	++
	C32	+
	C33	-
	C34	-
	C41	+++
	C42	-
	C43	-
	C51	-

Keys: + = Minimal growth, ++ = Medium growth, +++ = Maximum growth, - = No growth

Table 3: LDPE degradation by the fungal isolates

S. No. LDPE	Isolates	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	Percentage (%)
1	*Aspergillus niger*	17.0	13.4	3.6	21.1
2	*Aspergillus flavus*	18.6	15.6	3	16.1
3	Brown rot	9.1	7.5	1.6	17.6
4	white rot	10.4	8.5	1.9	18.3
5	Control	5.4	5.4	0	0

Surface Features of LDPE Film under SEM

Scanning Electron Microscopy to observe the surface morphology before and after biodegradation was carried out. Scanning electron micrographs of LDPE film before and after the treatment provided a direct and incontrovertible evidence of fungal activity on the LDPE film. Before the treatment, surface of the film appeared absolutely clean and smooth. However, post-treatment micrographs show variable intensity of fungal growth on the films where mycelia and conidia could be seen physically associated with the surface. The topography images of the treated films showed formation of grooves, corrosions, cracks and pits, etc. These images depict attachment and growth of *Alternaria alternate*, *Aspergillus niger* and *Aspergillus flavus*.

FTIR Analysis

The isolated fungal species secretes an enzyme during the biodegradation of polyethylene bags, which starts the oxidation reduction reaction, lysis of bonds, and, more precisely, esterification and some geometrical changes to the plastic bags. These changes may alter the low-density polyethylene bags' internal molecular structure. Fourier transform infrared spectroscopy analysis was utilized to identify the alteration in the internal chemical composition. For the control sample, the peaks at 2915 cm-1 and 2845 cm-1 showed C–H bond stretching. Furthermore, it was established that the peak at 717 cm-1 was caused by the CH2 rock vibrational mode and that the peak at 1464 cm-1 represented CH2 bent bonds.

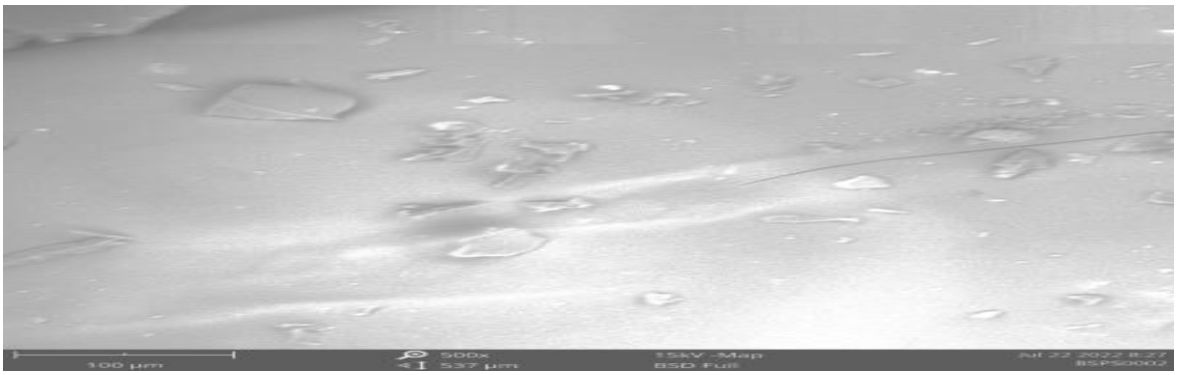

Figure 1. Surface Features of low-density LDPE film under SEM before biodegradation

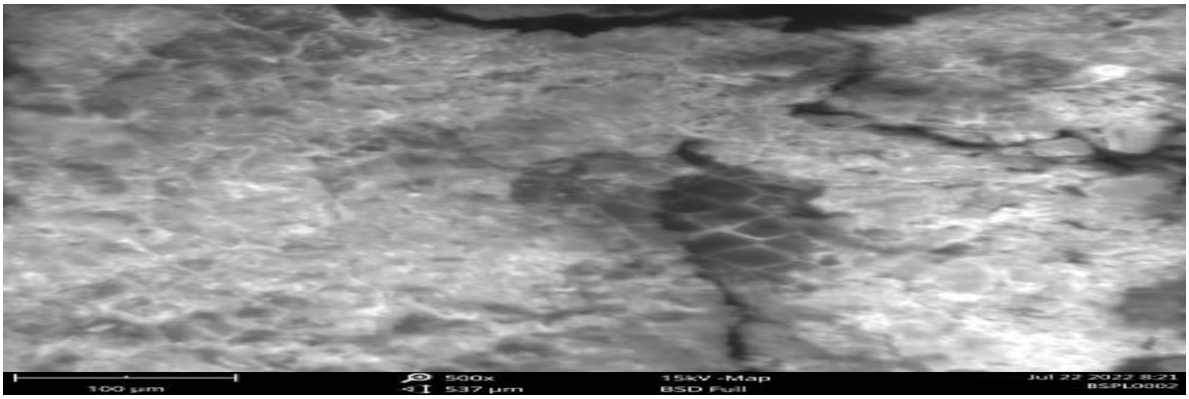

Figure 2. Surface Features of low-density LDPE film under SEM after biodegradation

Table 4. FTIR Analysis

Peaks	Variation Type	Functional Groups
2915	Stretching	C-H
2845	Stretching	C-H
1464	Bending	CH_2
717	Vibrating	CH_2

DISCUSSION

Numerous research have examined the employment of microorganisms in the biodegradation of LDPE over the years. Few research have used fungal species in controlled situations, despite the fact that many have used bacterial cultures to study the biodegradation of LDPE in natural settings. In the current study, LDPE was biodegraded in a lab setting using a fungus sample that included *Aspergillus niger* and *Aspergillus flavus*.

Since they can adhere to the surface of the substrate and produce a wide variety of enzymes under extremely diverse conditions, fungi are thought to be very powerful candidates for the biodegradation of plastic materials. This result indicates unequivocally that degrading fungi developed around the LDPE film, a phenomenon that might not have happened in the absence of a strong bond between the fungal cells and the substrate surface. Furthermore, the fungus has been consuming the film, which is only possible after an appropriate breakdown of the film material, as evidenced by the abundant proliferation of the active fungi surrounding the film in the absence of any carbon source in the broth. The nearly nonexistent growth of inocula under control circumstances lends even more credence to this conclusion. It has been successfully shown by Pramila and Ramesh (2015) that degrading *Aspergillus* organisms adhere to LDPE film in cultures. Six isolates' inocula eventually formed associations with the film throughout the fungus treatment of LDPE, and in 15 to 30 days, these associations grew to create colonies with varying sizes and appearances. These isolates included *Aspergillus niger* and *Aspergillus flavus*, which were suggested to be active on LDPE.

Using a microbalance, the weights of the samples both before and after degradation were determined. The weight reduction outcomes in mineral salt medium were noted over a duration of thirty days, correspondingly. After being incubated with the corresponding isolate for 30 days, *Aspergillus niger*, *Aspergillus flavus*, brown rot, and white rot fungi demonstrated reductions in the polyethylene of 21.1%, 16.1%, 18.3%, and 17.6% in the biodegradation experiment. In contrast, the control group did not exhibit any weight loss.

As to Varjani (2017), there was a decrease in the weight loss of LDPE over a 90-day incubation period, as the weight loss of polymer is typically related to the rate of biodegradation. After 90 days of incubation, the fungal isolates *Fusarium* sp. isolate PS3, *Penicillium* sp. isolate PS2, and other *Aspergillus niger* isolate PS3 showed weight loss of 0.59%, 0.36%, and 0.35%, respectively, while the control group showed no weight loss. The study on the biodegradation of LDPE under laboratory conditions was conducted by Varjani (2017). The one-way Analysis of Variance of fifty replicates and two trials revealed significant differences in the mean values between the control and *Aspergillus niger* isolate PS3 ($P=0.000$), control and *Fusarium* sp. isolate PS3 ($P=0.031$), and control and *Penicillium* sp. isolate PS2 ($P=0.010$).

However, this study's weight loss metrics are different from those of earlier research. It can primarily be the result of different protocols and insufficient fungal concentration during the incubation stage. Since the majority of early research focused on the biodegradation of LDPE utilizing broth cultures, solid plate culture technique was used in this study.

Pramila and Ramesh (2011) have published scanning electron micrographs of Aspergillus species of marine origin growing in combination with LDPE film. The Aspergillus species growing on LDPE film were shown in SEM photos; nevertheless, the fungal genotypes were separated from solid waste that was dispersed throughout contaminated areas. SE micrographs showing the LDPE film's disturbed surface and the hyphae's penetration into the film following Aspergillus species treatment combined with UV light. utilizing Aspergillus niger and Aspergillus flavus ATCC strains. Additionally, it was noted that the surface features of LDPE film treated with a microbial combination underwent significant alterations and erosion. They have also seen a decrease in the treated film's tensile strength. Findings from the current study and other studies strongly imply that certain fungi are able to solubilize the LDPE surface matrix and use it as a growth medium.

FT-IR analysis was performed on LDPE control and biodegraded samples. The polyethylene sample's deterioration was verified by FT-IR analysis, which also showed the structural differences between the control and degraded samples. The transmittance vs wave-number curves derived from the FT-IR analysis of samples that were stored in different mediums for control and degradation. For the control sample, the peaks at 2915 cm-1 and 2845 cm-1 showed C–H

bond stretching. Furthermore, it was established that the peak at 717 cm-1 was caused by the CH2 rock vibrational mode and that the peak at 1464 cm-1 represented CH2 bent bonds. Both of these outcomes are generally in line with the findings of Nourollahi *et al*. (2019).

A substantial decline in peak intensity was seen in the damaged samples over 20, 30, and 55 days, with wavenumbers equal to 2915 cm-1, 2845 cm-1, 1464 cm-1, and 717 cm-1. Varjani's (2017) research indicates that this kind of decline is indicative of biodegradation. After 55 days, the FT-IR curves showed that these peaks' strength had drastically decreased to the point where they are essentially non-existent and just barely distinguishable. Accordingly, a plot of absorbance vs wavenumber would show that the peaks at the aforementioned wave numbers have increased in peak intensity.

CONCLUSION

The study concludes that the fungal species play a significant role in the degradation of synthetic plastic, which can be used in future studies for the degradation of complex plastic materials. Finally, the study recommends that the fungal species of *Aspergillus niger* and *Aspergillus falvus* be considered as potential candidates to degrade LDPE.

REFERENCES

Abd El-Rahman, K. M., Abdellah Ali, S. F., Khalil, A. I., & Kandil, S. (2020). Influence of poly (butylene succinate) and calcium carbonate nanoparticles on the biodegradability of high density-polyethylene nanocomposites. *Journal of Polymer Research*, *27*, 1-21.

Awasthi, S., Srivastava, N., Singh, T., Tiwary, D., & Mishra, P. K. (2017). Biodegradation of thermally treated low density polyethylene by fungus Rhizopus oryzae NS 5. *3 Biotech*, *7*(1), 1-8.

Baculi, R, Q., Melegrito, J, B., Sabaten, R, D, S., and Caranto, M, R. (2017). "Biodegradation of high-density polyethylene by bacteria isolated from root nodules of Phaseolus vulgaris, Philipp. Agric. Sci.,100, 21–31.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries part 2*. Second Edition, Cambridge University Press UK. Pp 97-104, 132-142 and 178-180.

Kumari, S., Ramesh, D., Karthikeyan, S., Mutanda, T., Anandraj, A., & Bux, F. (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresource technology*, *102*(1), 57-70.

Nourollahi, A., Sedighi-Khavidak, S., Mokhtari, M., Eslami, G., & Shiranian, M. (2019). Isolation and identification of low-density polyethylene (LDPE) biodegrading bacteria from waste landfill in Yazd. *International Journal of Environmental Studies*, *76*(2), 236-250.

Paço, A., Duarte, K., da Costa, J, P., Santos, P, S, M, R., Pereira, Pereira, M, E., Freitas, A, C., Duarte, A, C., and Rocha-Santos, T, A, P. (2017). Biodegradation of polyethylene microplastics by the marine fungus Zalerion maritimum, Sci. Total Environ., 20-27.

Pramila, R., & Ramesh, K. V. (2015). Potential biodegradation of low-density polyethylene (LDPE) by Acinetobacter baumannii. *African Journal of Bacteriology Research*, *7*(3), 24-28.

Rajeswari, P., Samuel, P., Vijayakumar, J., Selvarathinam, T., Sudarmani, D. N. P., Amirtharaj, K., & Deenathayalan, R. (2017). Green synthesis of silver nanoparticles by Aspergillus consortium and evaluating its anticancer activity against Human breast adenocarcinoma cell line (MCF7). *Pharm. Biol. Eval*, *4*(1), 28-36.

Sangale, M. K., Shahnawaz, M., & Ade, A. B. (2019). Potential of fungi isolated from the dumping sites mangrove rhizosphere soil to degrade polythene. *Scientific reports*, *9*(1), 1-11.

Sindujaa, P., Padmapriya, M., Pramila, R., & Ramesh, K. V. (2011). Bio-degradation of low-density polyethylene (LDPE) by fungi isolated from marine water. *Research Journal of Biological Sciences*, *6*(4), 141-145.

Varjani, S. J. (2017). Microbial degradation of petroleum hydrocarbons, Bioresource Technology. 35-48.

Verma, N., & Gupta, S. (2019). Assessment of LDPE degrading potential Aspergillus species isolated from municipal landfill sites of Agra. *SN Applied Sciences*, *1*(7), 1-10.