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Polyethylene degradation potential of *Comamonas testosteroni* PRC1 and *Pseudomonas* sp. PRC2 isolated from soil samples of plastic recycling centre, Lagos, Nigeria.

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Abstract

Polyethylene polymer with its advantages of being strong, light-weighted, and durable, however, possesses disadvantages such as resistance to biological degradation and harmful to the natural environment. Solid waste related problems pose threat to megacities like Lagos. In this study, pooled soil samples collected randomly from a plastic recycling centre in Lagos were plated on Minimal Salt Medium with polyethylene as sole carbon source and screened for polyethylene-utilizing bacteria. The total dissolved organic matter was determined using the TOC titrimetric method and Fourier Transform infrared spectroscopy (FTIR) analysis was used to check the functional groups changes of the Low Density polyethylene (LDPE) material during the degradation period. Two bacterial strains *Comamonas testosteroni* PRC1 and *Pseudomonas* sp. PRC2 able to grow on polyethylene were used to degrade polyethylene. The persistence of the two isolates in the liquid medium after three months of incubation showed their ability to utilize the polyethylene material which served as carbon/ energy source for them. There was evolution of carbon (IV) oxide gas from the degradation experiment which was highest (55.50 ppm) at the 30th day with *Pseudomonas* sp. PRC2; the FTIR revealed disappearances and formation of new peaks in relation to the control sample. Alkenyl C=C stretch with wave numbers 1471cm^{-1} and 1647cm^{-1} disappeared in the polyethylene samples treated with *Comamonas testosteroni* PRC1 and *Pseudomonas* sp. PRC2 while carbonyl group with wave number 1716 cm^{-1} disappeared in the polyethylene samples treated with a combination of the two bacterial strains. The isolates showed degradative ability on the polyethylene (LDPE) material and thus can be used for biodegradation of polyethylene waste materials in the environment.

Keywords: Polyethylene, utilization, recycle, biodegradation

Introduction

Plastic materials are strong, light-weight, and durable and thus are widely used in food, clothing, shelter, transportation, construction, medical, and recreation industries [1]. Polyethylene (PE) of high and low density is primarily used in product packaging as sheets and thin films. Despite their wide applicability, the main limitation to their use is the fact that polyethylene adversely affects the environment [2]. Plastic materials like polyethylene (PE) are the potential source of environmental pollution. Their presence in soil causes infertility of soil, preventing degradation of other biodegradable substances, and also presents danger to animal life. The durability, light weight, and process-ability of polyethylene cause it to linger in the nature for centuries and end up in landfills and/or natural water resources [3]. Polyethylene is one of the most abundant commercially produced synthetic polymers [4].With huge amounts of polyethylene getting accumulated in the environment; the disposal evokes a big ecological issue and it takes thousand years for efficient degradation of polyethylene [5]. Its recalcitrant nature results from the high

molecular weight, complex three-dimensional structure, and hydrophobic nature, all of which interfere with its availability to microorganisms [6]. Reports on the biodegradation of polyethylene include that of Breslin [7] and Breslin and Swanson [8] but such results were based on PE blend with starch [9]. Biodegradation resulting from the utilization of polyethylene as a nutrient may be more efficient if the degrading microorganism forms a biofilm on the polyethylene surface. However, the hydrophobicity of the polyethylene interferes with the formation of a microbial biofilm. Watanabe et al. [10] isolated and identified three types of low-density polyethylene (LDPE) degrading microbes, namely; *Bacillus circulans*, *Bacillus brevis*, and *Bacillus sphaericus*, by soil burial method. Nanda and Sahu [11] investigated the biodegradation ability of *Brevibacillus, Pseudomonas*, and *Rhodococcus* spp. in degrading polyethylene. Also, Yang et al. [12] isolated two bacterial strains (*Enterobacter asburiae* YT1 and *Bacillus* sp. YP1) capable of degrading PE from waxworm's gut, while Gajendiran et al. [4] investigated biodegradation of LDPE using *Aspergillus clavatus* strain JASK1 isolated from landfill soil. In this study, two bacterial strains *Comamonas testosteroni* PRC1 and *Pseudomonas* sp. PRC2 capable of adhering to Low density Polyethylene (LDPE) pieces were isolated from soil samples of plastic recycling center. They were further tested for ability to utilize PE as sole carbon source and thus used to degrade the polyethylene polymer.

Materials and methods

Sample collection: Soil samples were collected randomly using soil auger up to a depth of 30-60cm from a plastic recycling centre into sterile polyethylene bag, pooled together and transported to the Environmental and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Nigeria for microbiological analysis.

Pulverization of PE: The PE material used as sole carbon source was pulverized using an electric blender, thus creating large surface area for action of microorganisms.

Enrichment technique for isolation of Polyethylene utilizing bacteria: This was carried out using the modified method of Baxi and Shah [13]. This technique was used to create a suitable environment for PE utilizing organisms in the soil samples. Ten gram of the soil sample was added to 90ml of Minimal salt medium consisting g/100ml of K_2HPO_4 0.1, KH_2PO_4 0.2, NaCl 0.05, $MgSO_4$ 0.2 and Pulverized PE 0.3 as an enrichment medium. This was incubated on the rotary shaker incubator (180 rpm) in an Environmental shaker incubator (G24, New Brunswick Scientific Co; Inc. Edison, N.J. U.S.A) at 30^oC for 5 days. After the 5^{th} day of incubation in the enriched medium, 1ml of the culture was serially diluted and plated out using the appropriate dilutions on Nutrient Agar and on a Synthetic Minimal Salt Agar of Baxi and Shah [13] containing g/l of: KH_2PO_4 0.2, K_2HPO_4 0.6, NaCl 0.3, NH_4SO_4 0.4, $MgSO_4$ 0.2, $CaCl_2$ 0.1, $FeCl_3$ 0.1, Polyethylene powder 5, Agar 15.

Identification of bacterial strains: These bacterial strains were identified as previously reported [14].

Inoculum preparation: The inoculum was prepared by culturing the bacteria isolates in Nutrient broth on a G24 Environmental incubator shaker (New Brunswick scientific Co; Inc. Edison USA) at 180 rpm for 48 hrs; cells were harvested by centrifugation at 7,000 rpm for 10 minutes, washed with and suspended in normal saline solution for use in the degradation experiment.

Polyethylene utilization and Measurement of biodegradation: The ability of the isolates to utilize polyethylene was tested by their inoculation into the MSM broth containing PE as the sole carbon source. The biodegradation ability was monitored on the basis of growth rate (optical density) using spectrophotometer (Camspec M105, China), change in pH, dissolved organic matter using the Total Organic Carbon (TOC) titration method (15) and functional group changes using the Fourier Transform Infrared (FTIR) spectroscopic analysis (16) of the control and treated experiment. FTIR was performed using Spectrum 400 IR system (8400S, Shimadzu, Japan). The entire spectral region between 400 and $4000cm^{-1}$ was scanned with a resolution of $4cm^{-1}$ for the control and bacteria-treated PE samples.

Results and discussion

The total heterotrophic count after enrichment for 5 days was $1.98x10^6$ cfu /ml, while colonies that grew on the Minimal salt agar (Polyethylene agar- PEA) were $1.70x10^4$ cfu/ml. The two bacteria isolates *Comamonas testosteroni* PRC1 and *Pseudomonas* sp. PRC2 grew on the PEA and were used for the biodegradation of PE. Fig.1 shows the pH changes recorded in the minimal salt medium over a sixty-day period at 15 days interval. It was observed that at the 15^{th} day, the pH of the MSM with the isolates *Comamonas testosteroni* PRC1 and *Pseudomonas* sp. PRC2 increased from 7.5 to 9.2 and it later dropped to 8.0 on the 30^{th} day and remained at 8.5 at both 45^{th} and 60^{th} day of the experiment. This was also the case when the two isolates were combined

while the control experiment did not show the same pattern of pH changes. This initial increase in the pH values may be because of the ammonification of nitrogen components as observed by Sahebnazar et al. [17]. This decrease and increase in pH is similar to that reported in a study by Jakubowicz et al. [18] in which the biodegradation of thermally oxidized biodegradable LDPE in soil for a period of 606 days was evaluated and also in a study by Esmaeili et al. [15].

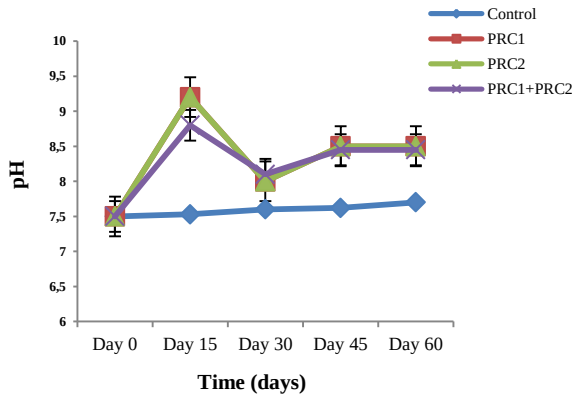

Figure 1. The pH changes of the MSM as the degradation progresses. Each data point represents the average of two replicates

The changes in the optical density (OD 600nm) on the growth of the organisms over sixty day period is shown in Fig.2. The growth rate for the isolates peaked at day 30 of the incubation period with *Pseudomonas* sp. PRC2 having the highest growth followed by *Comamonas testosteroni* PRC1 and then the combination of the two isolates. It was observed that when the isolates were cultured together, there seems not to be a synergestic effect even in the growth studies as their OD was lower than the individual ODs.

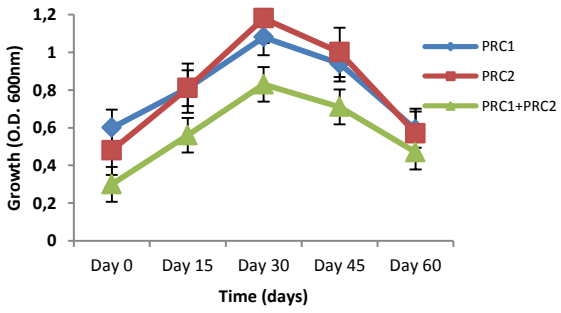

Figure 2. The growth measurements of the bacterial isolates using the optical density at 600nm. Each data point represents the average of two replicates

The Total Organic Carbon (TOC) in ppm of the isolates over the sixty-day period is shown in Fig.3. Biodegradation of polymer chains led to evolution of carbondioxide (CO_2). The evolved CO_2 in the treated samples were more than that recorded in the control. The TOC was at its peak on day 30 when the isolates *Pseudomonas* sp. PRC2 (55.50 ppm) and *Comamonas testosteroni* PRC1 (55.00 ppm) had the highest volume of CO_2 evoled from the degradation study but when the isolates were combined (*Comamonas testosteroni* PRC1 + *Pseudomonas* sp. PRC2), the highest volume of CO_2 was evolved at day 15 (54.40 ppm) after which it decreased. This increase in evolved CO_2 is similar to that reported by Esmaeili et al. [15] where the treatments inoculated with selected microorganisms demonstrated highest CO_2 production after 126 days.

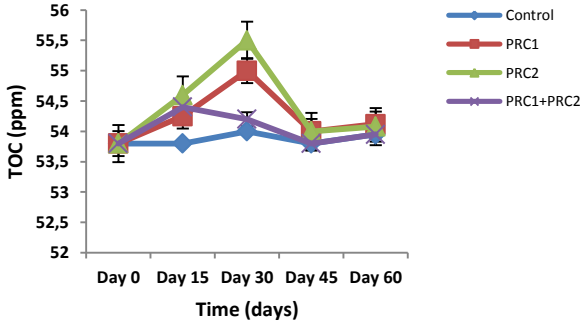

Figure 3. The total organic carbon (TOC) measurements used to monitor dissolved organic carbon as degradation progresses. Each data point represents the average of two replicates

The FTIR spectra analysis used to monitor the functional groups changes of the polyethylene material during the biodegradation study are shown in Figs. 4-7. From the FTIR analysis, it was observed that *Comamonas testosteroni* PRC1, *Pseudomonas* sp. PRC2 and a combination of the two isolates (PRC1 + PRC2) were able to degrade the LDPE material as there were disappearances of some peaks relative to the control sample, and also formation of new peaks were noticed in the degraded samples. This finding is consistent with the study of Esmaeili et al. [15], where the spectra of the film incubated in soil showed several new bands. Similarly, Das and Kumar [16] observed the formation of new and disappearance of some functional groups in their LDPE degradation studies by *Bacillus amyloliquefaciens*. The transmittance (%) in the treated samples was drastically reduced as compared to the control sample.

Some of the new peaks observed in LDPE sample treated with *Pseudomonas* sp. PRC2 were at

815cm^{-1} which was a 1,4 di-substitution (para) and 929cm^{-1} for 1,3 di-substitution (meta). Some of the new peaks observed in LDPE sample treated with *Comamonas testosteroni* PRC1 were at 1772-1869cm^{-1} for aromatic combination bands, and 1215cm^{-1} for aromatic C-H in plane bend. These new absorption bands of the spectra are possibly due to the oxidized fractions, such as moieties containing –OH groups, resulting from biodegradation of the PE samples by the isolates as opined by Corti et al. [19]. Some of the old peaks that disappeared during biodegradation included peaks at wave numbers 1471cm^{-1} and 1647cm^{-1} for Alkenyl C=C stretch which were observed in the LDPE samples treated with the bacterial strains singly and in combination.

Peak 1716 cm^{-1} for carbonyl group disappeared in the samples treated with *Pseudomonas* sp. PRC2 and a combination of the two isolates. These observations in the appearance and disappearance of peaks are attributed to the breaking down of the PE samples by the isolates. This is in line with the findings of Mohan et al. [20] that reported the use of the formation or disappearance of carbonyl groups and double bonds to elucidate the mechanism of biodegradation process in a study with fungal species. Also, Balasubramanian et al. [21] reported the isolation of some bacteria from the marine environment, for the degradation of HDPE and confirmed degradation using FTIR analysis of the samples.

Some of the peaks generated were reduced as a result of breakdown of the polymer chain. The peaks 420.5cm^{-1}, 1379.15cm^{-1} and 1543.1cm^{-1} were shifted to 418.57,1375.29 and 1541.18 cm^{-1} respectively by *Comamonas testosteroni* PRC1, while peaks 420.5, 669.32, 1543.1 and 2360.95 cm^{-1} were also shifted to 418.57,667.39,1541.18 and 2359.02 cm^{-1} respectively by *Pseudomonas* sp. PRC2. These observations correlate with the work of Mohan et al. [20] that observed reductions in peak 1718 cm^{-1} to 1715.33, 1715.41 and 1716.42 cm^{-1} respectively after microbial treatment which was attributed to the consumption of the carbonyl group by the microorganisms indicating the breakdown of polymer chain. The bacterial isolates used in this study were capable of utilizing PE without oxidation pretreatment and pro-oxidant additives; this is in contrast to reports of Sahebnazar et al. [17] and Sudhakar et al. [22] in which microorganisms could assimilate only the products of pre-oxidized PE but similar to the study of Esmaeili et al. [15] where their selected bacterial and fungal isolates utilized LDPE without pretreatments.

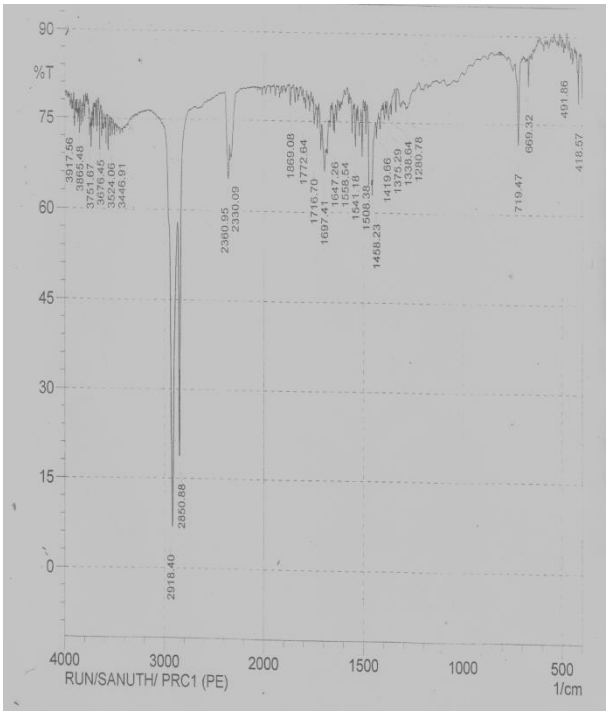

Figure 4. FTIR spectra of polyethylene sample degraded by PRC1

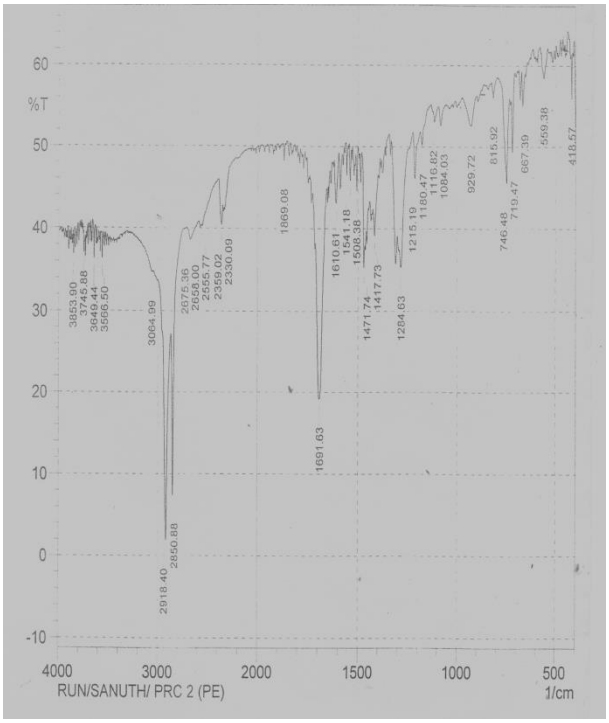

Figure 5. FTIR spectra of polyethylene sample degraded by PRC2

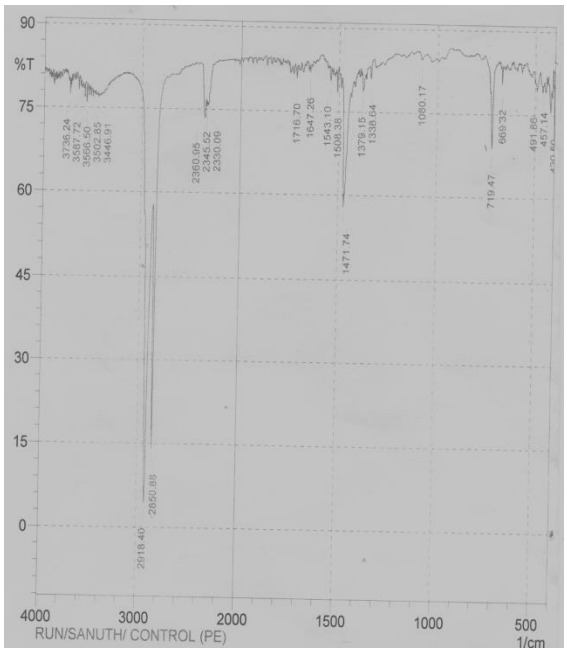

Figure 6. FTIR spectra of Control polyethylene sample

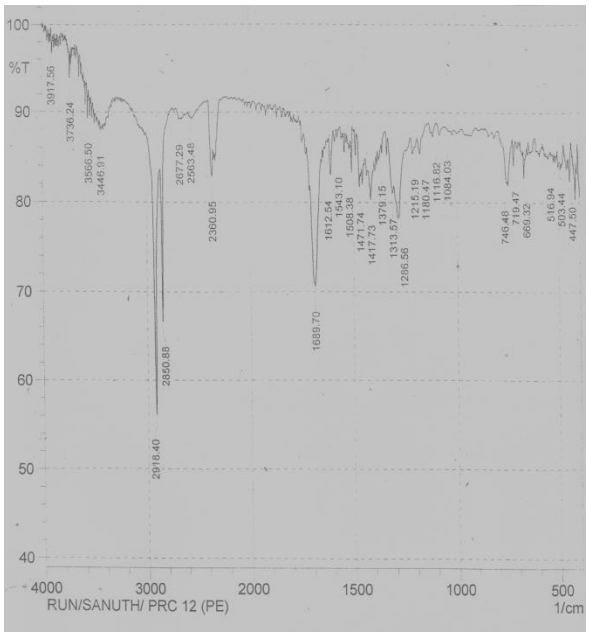

Figure 7. FTIR spectra of polyethylene degraded by PRC1and PRC2

The isolated bacteria were native to the site of polyethylene disposal and showed some degradability of the polyethylene in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media. This gives some clue that these bacteria can be used in both natural and artificial conditions for the purpose of degradation of polymers. Although there have been numerous investigations in degrading polyethylene, yet the fate of these organic polymers in the environment and the time required for their complete mineralization to carbon dioxide needs to be fully understood [23]. There is a growing interest in examining the activity of a consortium of microorganisms to expedite the biodegradation rate. Thus, further work is being done on the isolates.

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References

[1] Orhan, Y and Buyukgungor, H. 2000. Enhancement of biodegradability of disposable polyethylene in controlled biological soil. *Int. Biodeterior. Biodegrad*. 45: 49-55.

[2] Satlewal, A.; Soni, R.; Zaidi, M.; Shouche, Y and Goel, R. 2008. Comparative biodegradation of HDPE and LDPE using an indigenously developed microbial consortium. *J. Microbiol. Biotechnol*. 18.3: 477-482.

[3] Jang, J.C, Shin, P.K, Yoon, J.S, Lee, I.M, Lee, H.S. and Kim, M.N. 2002. Glucose effects on the biodegradation of plastics by compost from food garbage. *Polymer Degradation and Stability* 76:155-159.

[4] Gajendiran, A.; Krishnamoorthy, S. and Abraham, J. 2016. Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. 3 *Biotech*. 6. (1): 52

[5] Shah, A.A; Hasan, F; Hameed, A and Akhter, J.I. 2009. Isolation of *Fusarium* sp. AF4 from sewage sludge, with the ability to adhere the surface of polyethylene. *African Journal of Microbiology Research* 3.10: 658-663.

[6] Hadad, D.; Geresh, S and Sivan, A. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol*. 98: 1093-1100.

[7] Breslin, V.T .1993. Degradation of starch-plastic composites in a municipal solid waste landfill. *J. Environ. Polym. Degrad*. 1: 127-141.

[8] Breslin, V.T and Swanson, R.L. 1993. Deterioration of starch-plastic composites in the environment. *J. Air Waste Manag. Assoc*. 43: 325- 335.

[9] Pometto, A.L.; Johnson, K.E and Kim, M. 1993. Pure-culture and enzymatic assay for starch-polyethylene degradable plastic biodegradation with *Streptomyces* species. *J. Environ. Polym. Degrad.* 1: 213-221.

[10] Watanabe, T.; Ohtake, Y.; Asabe, H.; Murakami, N and Furukawa, M. 2009. Biodegradability and degrading microbes of low-density polyethylene. *J. Appl. Polym. Sci*. 111: 551-559.

[11] Nanda, S and Sahu, S.S. 2010. Biodegradability of polyethylene by *Brevibacillus, Pseudomonas* and *Rhodococcus* spp. *New York Science Journal* 3.7:95-98.

[12] Yang, J.; Yang, Y, Wu, W., Zhao, J. and Jiang, L. 2014. Evidence of Polyethylene Biodegradation by Bacterial Strains from the Guts of Plastic-Eating Waxworms**.** *Environ. Sci. Technol.,* 48 (23):13776–13784

[13] Baxi, N.N and Shah, A.K. 2002. ε-Caprolactam-degradation by *Alcaligenes faecalis* for bioremediation of wastewater of a nylon-6 production plant. *Biotechnology letters* 24: 1177-1180.

[14] Fagade, O.E; Sanuth, H.A; Okolie, B.I; Yogesh, S and Amit, Y. 2012. Molecular Characterization and Phylogenetic Analysis of *Comamonas testosteroni* and *Pseudomonas* sp. Isolated from Soil Sample of a Plastic Recycle Centre. *Waste Management and Bioresource Technology* 2: 1-7.

[15] Esmaeili, A., Pourbabaee, A.A, Alikhani, H.A, Shabani, F. And Esmaeili E. 2013. Biodegradation of low density polyethylene (LDPE) by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in soil. *PLoS ONE* 8(9): e71720. doi:10.1371/journal.pone.0071720

[16] Das, M.P. and Kumar, S. 2015. An approach to low density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *3 Biotech.* 5:81–86. doi: 10.1007/s13205-014-0205-1.

[17] Sahebnazar Z, Shojaosadati SA, Mohammad-Taheri M, Nosrati M .2010. Biodegradation of low-density polyethylene (LDPE) by isolated fungi in solid waste medium. *Waste Manage* 30: 396–401.

[18] Jakubowicz I, Yarahmadi N, Arthurson V. 2011. Kinetics of abiotic and biotic degradability of low-density polyethylene containing pro-degradant additives and its effect on the growth of microbial communities. *Polym Degrad Stab* 96: 919–928.

[19] Corti A, Muniyasami S, Vitali M, Imam SH, Chiellini E. 2010. Oxidation and biodegradation of polyethylene films containing pro-oxidant additives: Synergistic effects of sunlight exposure, thermal aging and fungal biodegradation. *Polym Degrad Stab* 95: 1106–1114.

[20] Mohan, K.R.K; Kuruganti, S.A; Jakkula,.S.V; Rohini, K.D.B. and Lakshmi, M.N. 2010. Synergistic Effect of Chemical and Photo Treatment on the Rate of Biodegradation of High Density Polyethylene by Indigenous Fungal Isolates. *International Journal of Biotechnology and Biochemistry* 6.2:157–174

[21] Balasubramanian, V.; Ramesh, N.; Sumathi, C.S and Kannan, V.R. 2008. Isolation of Efficient Polyethylene Degrading Bacterial Species from Marine Ecosystem of Gulf of Manna in India. *J. Soil Sci. Plant Nutr*. 8.3: 268

[22] Sudhakar M, Doble M, Sriyutha Murthy P, Venkatesan R. 2008. Marine microbe-mediated biodegradation of low- and high-density polyethylene. *Int Biodeterior Biodegradation* 61: 203–213.

[23] El-Shafei, H.A.; El-Nasser, N.H.A; Kansoh, A.L. and Ali, A.M. 1998. Biodegradation of disposable polyethylene by fungi and Streptomyces species. *Polymer Degradation and Stability* 62:361-365.

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