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Full Length Research Paper

Occurrence and recalcitrance of polyethylene bag waste in Nigerian soils

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Attempts to biodegrade polyethylene bag wastes (pure water sachets) weighing 25.2 g each were made by burying them in the soil and subjecting them to acid treatment (0.5 M HNO3) and alkaline treatment (0.5 M NaOH) over a 24 week study period. The experimental polyethylene bags (ED1) were inoculated with a strain of Pseudomonas spp. isolated from a refuse dump and by inorganic nutrient supplementation to facilitate biodegradation. Samples without inorganic nutrient supplementation and Pseudomonas spp. inoculation served as first control (CD1), while those placed on a slab on the surface of the soil served as second control (CD2) to evaluate the roles played by microorganisms and physical degradation, respectively. ED1 showed a slight reduction in weight to 24.9 g at week 16 after the acid treatment for 5 days and further reduced to 24.7 g at the end of the study after the alkaline treatment. CD1 reflected no variations in weight, while CD2 reduced to 25.1 g. The study showed that recycling of used pure water sachets which yielded useful products such as water seal, polyethylene bags and jerry can covers, appears to be the only option of checking environmental pollution caused by this product as microbial degradation proved ineffective.

Key words: Biodegradation, polyethylene, waste, recycling, recalcitrance.

INTRODUCTION

Polymers are large molecules formed by the union of identical monomers which may be natural (Cellulose or DNA), or synthetic (nylon or polyethylene) (Encyclopaedia of Polymer Science and Technology, 1992). In earlier times, before the making of synthetic polymer, what the world used to know was rubber. Natural rubber, as it was then known, was of limited usefulness to the industries. Polyethylene is formed through addition polymerization reaction. This occurs by a sequential addition or linking of a large number of unsaturated molecules (the monomer unit – ethene). Polyethylene is a waxy, transculent, flexible thermoplastic.It is one of the lightest plastics having a specific gravity of 0.92 - 0.93. Below 60˚C, polyethylene is insoluble in all solvents and is resistant to the action of most chemicals other than strong oxidising acids. However, above 115˚C, the polymer changes from a clean solid to a low viscosity melt. At this temperature and above, exposure to air causes relatively extensive oxidative degradation, unless antioxidants are included in the polymer (Encyclopaedia of Polymer Science and Technology, 1992). A number of factors can initiate the degradation of polyethylene, such as ultra-violet light, heat, oxygen and film stress (such as pulling and tearing) (Scott, 1999). Polyethylene is probably the polymer seen most in the world. This is because polyethylene is cheap, safe, harmless, and stable in most environments and easily processed (Ojo, 2007). The use to which polyethylene is put depends on whether it is low-density or high-density type. Low- density polyethylene is used as kitchen utility ware, in the manufacture of toys, process tank liners, closure packages, sealing rings and battery parts. Other uses are as squeeze bottles for packaging and containers for drugs. The film is used as wrapping materials for food, fruits and clothes (Efiuvwevwere and Oyelade, 1991; Nwachukwu et al.,

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Abbreviations: ED1, Experimental design 1; **ED2,** experimental design 2; **NA,** nutrient agar; **CA,** cetrimide agar; **MCA,** MacConkey agar; **PDA,** potato dextrose agar; **EMBA,** eosin methylene blue agar; **DO,** dissolved oxygen; **BOD,** biochemical oxygen demand; **CD1,** first control designs; **CD2,** second control design.

2008).

Polyethylene is also used in covering wires and cables because of its high insulating properties. It is also moulded into pipes used in transporting chemicals, natural gas and water for various uses. High density polyethylene is used in making refrigerator parts, pipes, defroster, heater ducts, sterilizable house wire and hospital equipment and hoops. This is attributed to its higher resistance to high temperature. In Nigeria, waste disposal is very poor. Most of the polyethylene wastes originate from consumption of products packaged in polyethylene sachets, bags or other containers. Xenobiotics such as polyethylene are chemically synthesized organic compounds, most of which do not occur in nature (Ojo, 2007). They are compounds that are foreign to a living organism. Where these compounds are not easily recognized by existing degradative enzymes, they accumulate in soil and water (Esteve-Nenez et al., 2001; Ojo, 2007). Indiscriminate dumping of the so called 'pure water sachets' on roads, and drainage channels in Nigeria cause blockage of such channels and flooding of the environment during rainy season. In a bid to minimize these effects, burning of polyethylene waste materials has been adopted as a management strategy. This method in effect, constitutes a greater part of environmental pollution with many green house effects. Gases such as carbon dioxide, carbon monoxide and subsequently sulphur and nitrogen oxides are released into the atmosphere giving rise to acid rains, ozone depletion and global warming. Synthetic fibres like polyethylene and polypropylene are practically non-degradable. Although, more organisms are being described as being able to degrade these anthropogenic molecules, some xenobiotics have been shown to be unusually recalcitrant (Esteve-Nenez et al., 2001). The ability of Pseudomonas spp. to degrade organic pollutants has been reported in earlier studies (Nwachukwu et al., 1999; Nwachukwu, 2000). Pseudomonas spp. are known to have strong ability to resist toxic substances, including heavy metals such as mercuric compounds and disinfectants (Baron et al., 1994). Pseudomonas spp. are equally highly amenable to genetic manipulation, a desired attribute of microorganisms favoured in bioremediation. The problem of pollution caused by polyethylene waste materials cannot be overemphasised. The aim of this study was therefore, to develop a safe disposal method for polyethylene waste materials.

MATERIALS AND METHODS

Culture development

A strain of Pseudomonas spp. isolated from soil samples in a refuse dump was used for the study. The overnight pure cultures were harvested from nutrient agar (NA) plates and emulsified in nutrient broth. The tubes were incubated aerobically for 2 - 5 h to produce a bacterial suspension of moderate cloudiness. Growth was monitored tubidimetrically at 600 nm and by standard plate count.

The density of the broth was standardized by dilution with nutrient broth to a density of approximately 4.6 x 107 cfu/ ml which corresponded with the optical density of 1.17.

Study site

A small piece of land measuring 10 m2 in the Botanical garden of the University of Lagos, Nigeria was mapped out and divided into 4 extremes. Holes were dug at three of the sections to the depth of 20 cm and used for the biodegradation study.

Biodegradation procedure

Some polyethylene bags weighing 25.2 g each were collected and buried in soil at 20 cm depth. The bags were properly spread out and then inoculated with 250 ml of the developed culture containing Pseudomonas spp. to the level of 4.6 x 107 cfu/ml. The bags were slightly covered with the soil after inoculation with the developed culture. This was set-up in duplicates and designated as the Experimental Designs 1 and 2 (ED1 and ED2). Distilled water (250 ml) containing Raymond's inorganic nutrients was added to ED1 weekly to facilitate biodegradation. Similarly, another two systems designated as the Control Designs 1 and 2 (CD1 and CD2) were set-up: CD1 was set up just like the ED2 except that it was not inoculated with Pseudomonas spp. CD2 was the second control in which the polyethylene bags were placed on a slab on the surface of the soil without any other treatment.

Collection of samples

Immediately after setting up the experiment, and at intervals of 4 weeks, soil samples adhering to the polyethylene bags were aseptically collected from the set-ups and at the depth of 20 cm over a period of 24 weeks. The soil samples were immediately analyzed. The changes in weight of the polyethylene bags were determined at two week-intervals. Before designing the set-ups, the bags were weighed. At two weeks intervals, the bags were recovered from the soils and the slabs, rinsed in three clean water changes, air-dried and weighed. This was done for 8 weeks with no noticeable changes in mass of the bags. Furthermore, attempts were made to improve the biodegradability of the bags. To achieve this, the bags after recovering from soil, drying and weighing, were treated with 0.5 M HNO3 at week 8. The bags were separately placed in trays and 250 ml of 0.5 M HNO3 was poured into the trays. The bags were left there for 5 days after which they were recovered, washed and replaced in the soil with fresh inoculums. Also, changes in the mass of the bags were monitored as described above for another 8 weeks making the total time of sampling to be 16 weeks. At week 16, there was only a slight change in the weight of the bags, hence the next treatment which involved exposure to alkaline substance. To achieve this, the experimental polyethylene bags were recovered, as described above and now treated by immersing in trays containing 250 ml of 0.5 M NaOH for 5 days. At the end of this treatment time, the bags were rinsed and replaced in the soil with fresh inoculums of Pseudomonas spp. The samples were left for another 8 weeks to allow digestion to take place (that is, 24 weeks investigation). At the end of this period, the bags were recovered, rinsed, dried and weighed. During this experiment, biodegradation was also monitored by determining the mean changes in the total counts of Pseudomonas spp., other heterotrophs, (Figure 2 and 3) and in the concentrations of inorganic nutrients (nitrates) (Figure 6), pH, dissolved oxygen (DO) (Figure 4) (Singh et al., 1999), biochemical oxygen demand (BOD) (Figure 5) (Odiete, 1999) at intervals of 4 weeks over a period of 24 weeks.

Microbiological analysis

Soil samples (1 g) were transferred to a test tube containing 9 ml of

sterile distilled water to give 10-1 dilution, from which higher dilutions were made. Aliquots (0.1 ml) from 10-2, 10-4 and 10-6 dilutions of the soil samples were plated on cetrimide agar (CA), MacConkey agar (MCA), nutrient agar (NA), potato dextrose agar (PDA) and eosin methylene blue agar (EMBA). The CA, MCA, NA and EMBA plates were incubated aseptically at 37°C for 1 - 2 days for isolation of bacteria. PDA plates were incubated at room temperature (30+20C) for 3 - 6 days (Nwachukwu and Akpata, 2003). At the end of incubation, the colonies were counted. The relative abundance (population density) of the organism was estimated by multiplying the plate count per ml for each organism by the dilution factor used (Nwachukwu and Ugoji, 1995). In this study, the counts for bacteria, moulds and yeasts were summed up and reported as the total heterotrophs. Isolated colonies were identified using the taxonomic characteristics and methods outlined in the Bergey's Manual of Determinative Bacteriology for Bacteria (Barrow and Feltham, 1995); (Krieg and Holt (1984). Bergey's Manual of Systematic Bacteriology (1) Baltimore et al. (1986), Bergey's Manual of Systematic Bacteriology (2) Baltimore et al. (1969) for fungi.

Recycling

Polyethylene wastes (pure water sachets) were picked from different sources, rinsed and ground in a grinding machine. The product was then loaded into a hopper (bin where the material is collected) from where it was fed gradually into a mechanical system comprising of a melting zone, compression zone and a feeding zone. The materials got melted at about 150°C into a molten form and were passed into the compressing zone where air was applied and then made into a mould. The material assumed the shape of the mould and was cooled by an inbuilt water-cooling system. On cooling, pressure was applied hydraulically and a finished product emerged. The investigation showed that polyethylene wastes recycling method can be established easily with no adverse effect on the environment (Noritake et al., 2008).

Statistical analysis

The data obtained from the weight of the polyethylene bag samples, over the 24 week study period, were fitted to ANOVA One-Way Test for significance evaluation (Rangasamy and Venketraman, 2009).

RESULTS

The mean changes in the weight of the polyethylene bag samples during the 24 week study period are shown in Figure 1. There was no loss in weight of the initial 25.2 g of the samples subjected to biodegradation within the first 8 weeks. However, after 8 weeks, during the second stage of the experiment, which involved treatment with 0.5 M HNO3, a slight decrease in the weight of samples from 25.2 - 24.9 and 25.2 - 25.1 g was observed in ED1 and CD2, respectively. Furthermore, after treating the polyethylene bag samples with alkaline substance at week 16, the weight for both ED1 and CD2 decreased by 0.5 g. The result of statistical analysis indicated that the loss in weight of the samples was significant ($p < 0.05$) when treated with the acid and with the alkaline substance. The isolated organisms were identified as Pseudomonas aeruginosa, *Pseudomonas putida*, *Bacillus subtilis* and *Aspergillus niger*. Discolourations were also observed on the polyethylene bag samples. The samples in CD2 after a period of time lost the initial blue paint prints on them. The samples when treated with the acid had a colour change from colourless to pale blue while after treatment with alkaline solution turned purplish-blue. The mean changes in the population densities of Pseudomonas spp. and the total heterotrophs present, in the soil samples are shown in Figure 2 and 3, respectively. It can be deduced that the growth of Pseudomonas spp. and total heterotrophs in ED1 was less after treatment with acid than when treated with the alkaline solution. Generally, there were slight fluctuations in the dissolved oxygen content (DO) and pH profiles throughout the period in all the setups. ED1 registered lower levels of dissolved oxygen throughout the 24 week period than ED2 and the control design (Figure 4). The pH of the soil samples throughout the period was between 5.3 and 6.8. The recycling experiment yielded useful products, which included polyethylene bags, jerry can covers, water seal nail stoppers etc., depending on the shape of mould used. Some of these products are shown in Figures 7, 8 and 9. Figure 5 shows that the mean changes in biochemical Oxygen Demand (BOD) content of the soil samples decreased after acid and alkaline treatment in samples ED1 and CD1.

The BOD content of sample ED2 increased from 540 – 650 mg/kg within 4 weeks and thereafter was constant till the 20th week. The BOD for sample ED1 decreased from 680 – 550 mg/kg from week 0 - 8. After the acid treatment, it reduced further to 500 mg/kg. The BOD content of sample CD1 also increased from 400 mg/kg at week 0 to 580 mg/kg at week 8. Subsequent to the acid treatment, it decreased to 500 mg/kg. This was maintained till the 16th week. It is noteworthy to see that after the 16th week, the BOD content of CD1 increased sharply from 500 - 600 mg/kg but after the alkaline treatment, it reduced from 600 - 500 mg/kg. The mean changes in nitrate content of soil samples are shown in Figure 6. Sample ED1 had the highest increase in nitrate content after the alkaline treatment. This was followed by sample ED2. All the samples showed slight reduction in nitrate content after the acid treatment. The effectiveness of the acid treatment could be linked to the strong oxidizing properties of HNO3.

DISCUSSION

In this study, attempts were made to get polyethylene waste products biodegraded by treatment with strong acid and base, inorganic nutrient supplementation (biostimulation) and addition of a selected microorganism to augment the indigenous microflora. (The indigenous mocroflora were not excluded in the biodegradation procedure). In other words, by these treatments, all the materials required to support microbial growth as to stimulate degradation of the waste products were provided.

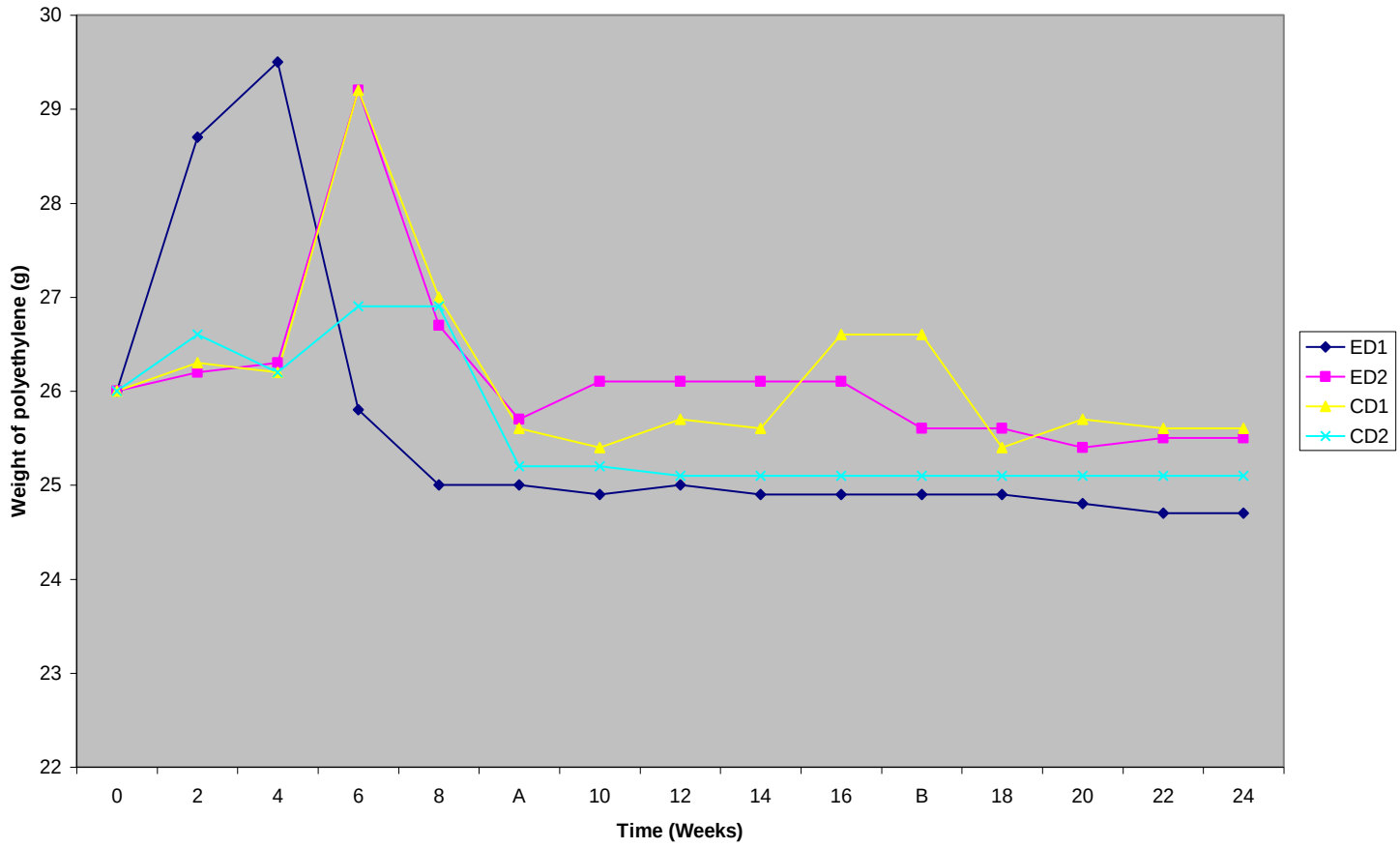

Figure 1. Mean changes in the weight of polyethylene bag samples during the 24 weeks study period. A After 5 days in 0.5M HNO_3; B, After 5 days in 0.5M NaOH; ED1, Polyethylene + *Pseudomonas* sp. + Raymond's medium in the soil; ED2, Polyethylene + *Pseudomonas* in the soil; CD1, Polyethylene in the soil; and CD2, Polyethylene on the surface of the soil (on a slab).

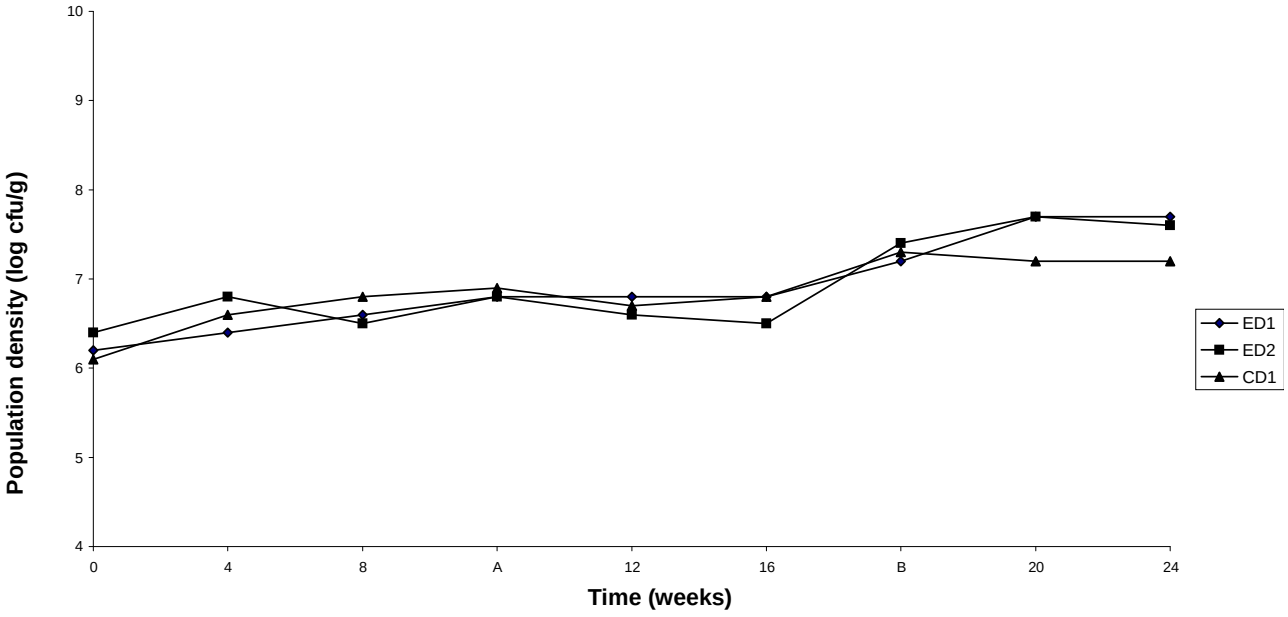

Figure 2. Mean changes in the population density of *Pseudomonas* sp. in the soil samples. A, After 5 days in 0.5M HNO_3; B, After 5 days in 0.5M NaOH; ED1, Polyethylene + *Pseudomonas* sp. + Raymond's medium in the soil; ED2, Polyethylene + *Pseudomonas* in the soil; CD1, Polyethylene in the soil; Polyethylene + *Pseudomonas* in the soil.

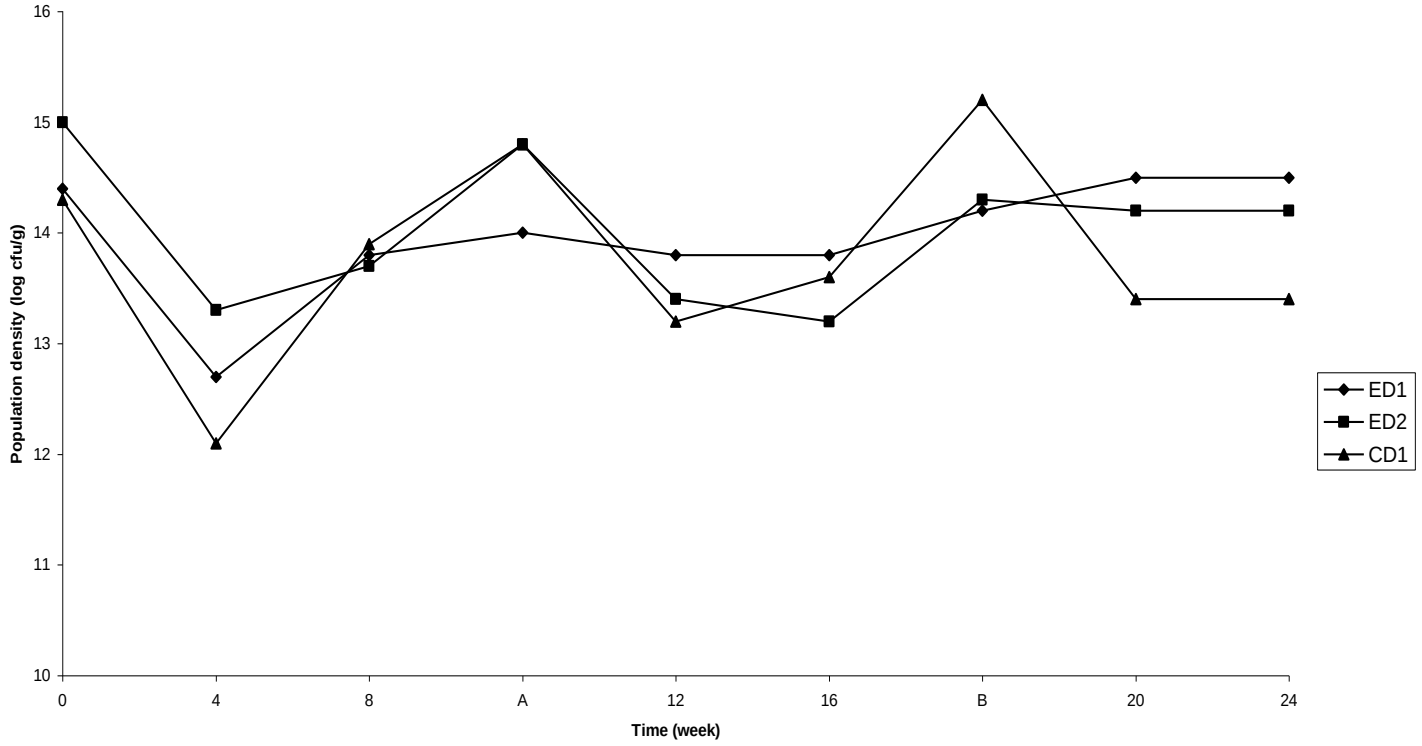

Figure 3. Mean changes in the population density of total heterotrophs in the soil samples. A, After 5 days in 0.5M HNO_3; B, After 5 days in 0.5M NaOH; ED1, Polyethylene + *Pseudomonas* sp. + Raymond's medium in the soil; ED2, Polyethylene + *Pseudomonas* in the soil; CD1, Polyethylene in the soil.

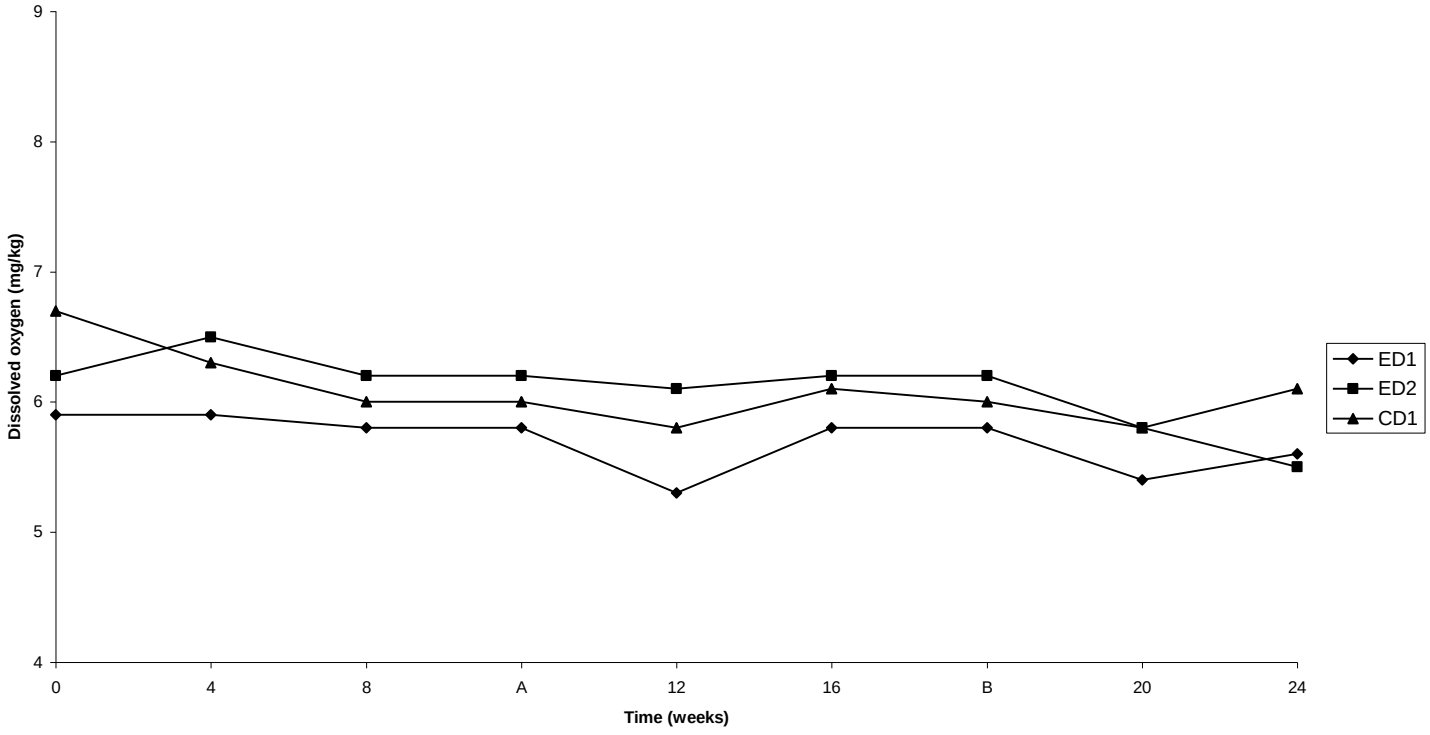

Figure 4. Mean changes in Dissolved Oxygen (DO) content of the soil samples. A, After 5 days in 0.5M HNO_3; B, After days in 0.5M NaOH; ED1,Polyethylene + *Pseudomonas* sp. + Raymond's mediuim in the soil; ED2, Polyethylene + *Pseudomonas* in the soil; CD1, Polyethylene in the soil.

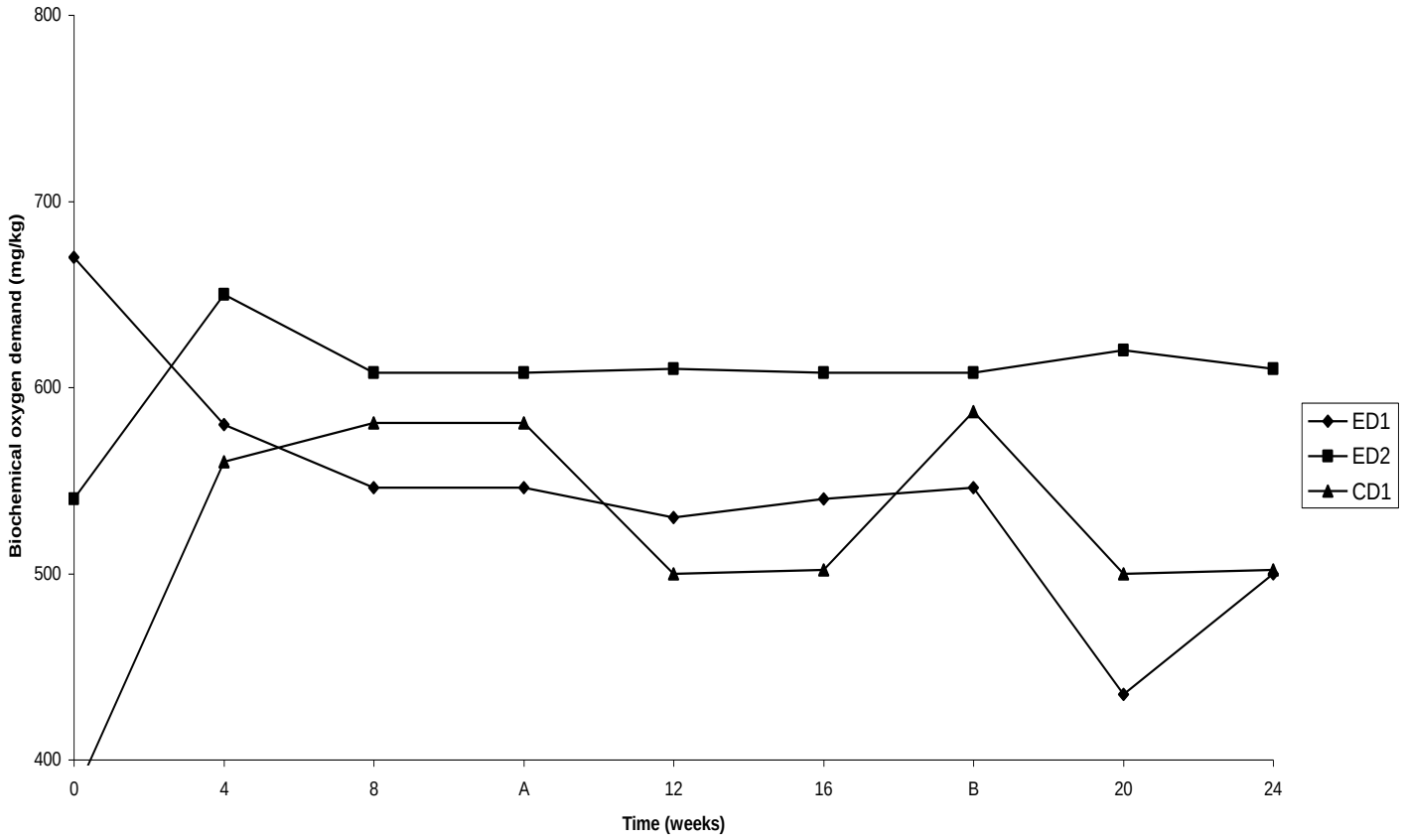

Figure 5. Mean changes in Biochemical Oxygen Demand (BOD) content of soil samples. A, After 5 days in 0.5M HNO_3; B, After 5 days in 0.5M NaOH; ED1, Polyethylene + *Pseudomonas* sp. + Raymond's medium in the soil; ED2, Polyethylene + *Pseudomonas* in the soil; CD1, Polyethylene in the soil.

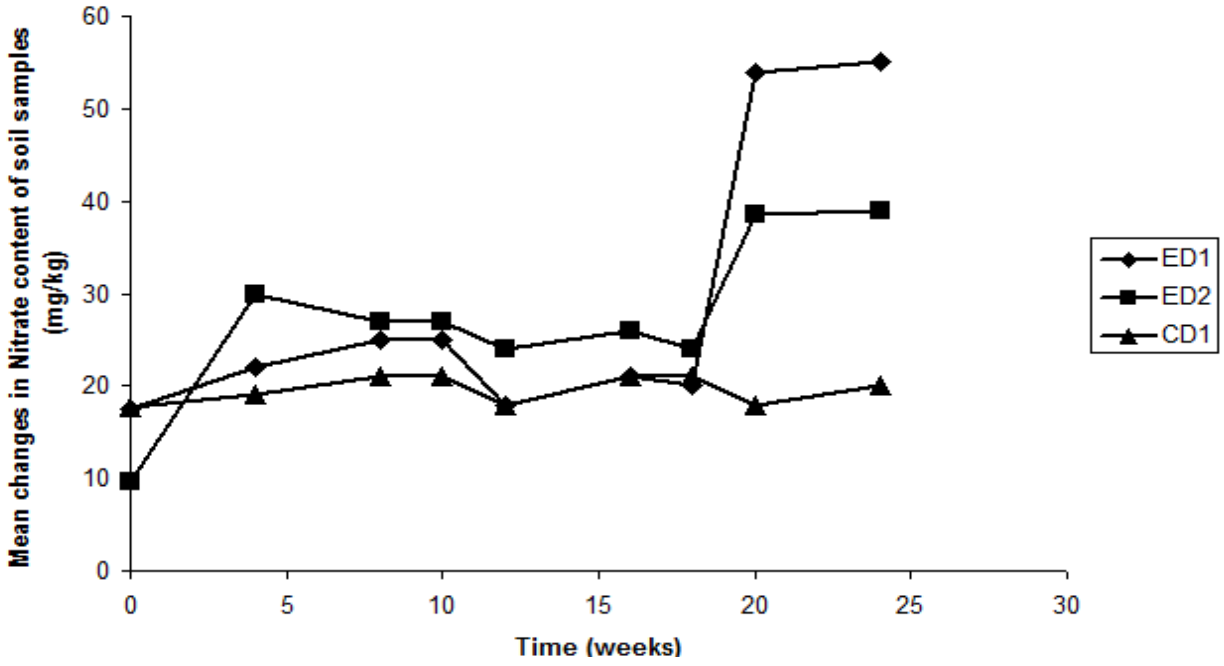

Figure 6. Mean changes in NO_3^- content of soil samples. ED1, Polyethylene + *Pseudomonas* sp. + Raymond's medium in the soil; ED2, Polyethylene + *Pseudomonas* in the soil; CD1, Polyethylene in the soil.

Figure 7. Recycled product (polyethylene carrier bag).

However, from the results of this study, these treatments did not bring about any significant change in the biomass of the waste products. The small change in biomass observed for the waste products could be attributed to the disappearance of the printing materials on the surfaces of the polyethylene products. Probably, the acid/alkaline treatments which have corrosive properties enhanced their being peeled off while some of the components are organic and utilisable by micro-organisms as sources of energy and carbon.

Polyethylene has long been known as a xenobiotic compound which is not easily biodegraded (Esteve-Nenez et al., 2001). This explains why they end up in landfills and dumpsites as recalcitrant pollutants. For biodegradation to be successful, in-depth knowledge about the types of organisms which mediate detoxification of the pollutant will help determine the process conditions that should be used in practical treatment systems (Obayori et al., 2008). This is accounted for in part by the fact of their possession of plasmid-mediated genes. Venter et al. (2004) using a cultivation-independent molecular approach, found thousands of new bacterial species with more than one million new protein-coding genes in Sargasso sea water. This suggests that there are millions of genes, uncharacterised micro-organisms and other protein-coding genes yet to be discovered, thus presenting a tremendous potential for the discovery of new xenobiotic degradation pathways. This is corroborated

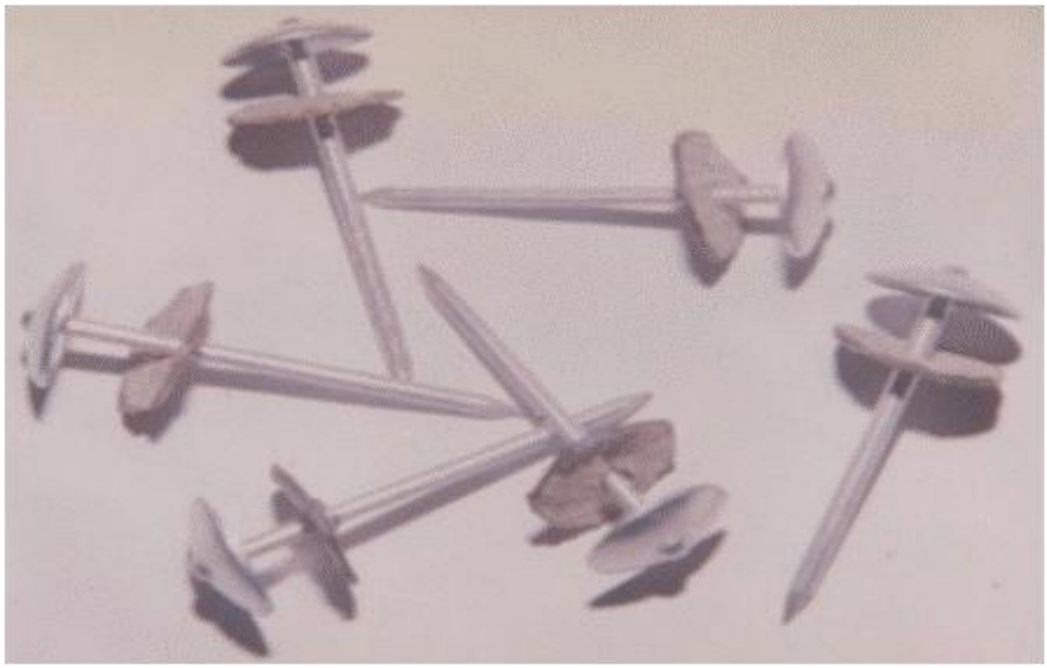

Figure 8. Recycled product water seal (nail stopper).

Figure 9. Recycled product (Jerrycan stoppers).

by the discovery of El-Fantroussi (2000) who reported similar observations from the study of biodegradation of Linuron, a herbicide where majority of the microbial species involved in the biodegradation were detectable only by denaturing Gradient gel electrophoresis (DGGE) because they were difficult to culture. Metagenomics, the culture-indepen-dent genomic analysis of entire microbial communities (Schloss and Handelsman, 2003) may provide an access to the pool of genomes in specific species which may be useful for future biodegradation procedures for polyethylene. Watanabe and Kawai (2006) studied the possibility for enzymatic degradation of xenobiotic polymers and described a mathematical model for depolymerization of polyethylene glycol.

Kawai et al. (2002) also reported the biodegradability of photodegraded polyethylene (PE) and biodegradation by

microbial consortia which were confirmed by the increase of viable cell number. By week 24, when this study was completed, no further decrease in mass of the waste products was observed. In other words, the small decrease in the biomass of the waste products could not be due to microbial action. This is because for the first eight weeks prior to treatment with acid and alkali, there was no loss in mass even though the waste products were exposed to microbial action. However, because microorganisms are ubiquitous and therefore have the potential of adapting to polyethylene as novel growth and energy substrates, the possibility exists that micro-organisms may be involved in slow degradation of polyethylene. It is however, noteworthy that a major problem of biodegradation of pollutants is that the inter-mediary products of degradation often become toxic to the organisms (Obayori et al., 2008). Thus from the results of this study, recycling of polyethylene and poly-ethylene product seems to be the most readily available option to clear the environments of the nuisance of these waste products. Useful products such as stoppers for plastic containers, roofing nails, water seal and fresh polyethylene bags or sachets were made from the recycled product. This strongly suggests that recycling of polyethylene wastes into useful products could be the possible option or method of disposal of polyethylene waste materials. This method of recycling if practised on a routine basis by manufacturers of polyethylene products will immensely eliminate this pollutant from the natural environment.

Conclusion

Manufacturers of polyethylene products are encouraged, from the results of this study to design mechanisms to retrieve their waste products and recycle them to form new, useful products. This will improve their profit margin while pollution of the environment with these waste products will be eliminated. Further studies may be required to investigate new xenobiotic degradation path-ways.

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