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A STUDY OF TECHNIQUES FOR SUSTAINING HEALTHY ENVIRONMENT THROUGH MICROBIAL DEGRADATION OF POLYETHYLENE AND PLASTIC POLLUTANTS

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ABSTRACT

Polythene and Plastic wastes range in types including pure water sachets, cellophane bags, plastic packaging of confectioneries, bakery products, toiletries, laminating materials, soft drinks, pieces of plastic buckets, plates, cups, wrappings etc. They are found in household wastes, refuse dump sites, municipal drainage system, street refuse collections, where they

Introduction

Biodegradation is defined as decomposition or destruction of contaminant molecules by the action of the enzymatic machinery of biological system. Biodegradation is the process by which organic substances are broken down by living organisms. The term is often used in notation to ecology waste management and environmental remediation. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process (Ali *et al*., 1998).

Microorganisms have natural potentials to degrade, transform or accumulate a huge range of compounds including hydrocarbons, pharmaceutical substances and metals. The durability, light weight and process ability of these polymers cause them to linger in nature for centuries and end up in landfills and natural water

resources creating a severe threat to the environment and its ecosystems (Hoffmann *et al*., 2003).

Biodegradable polymers are designed to degrade upon disposal by the action of living organisms, biodegradable polymers generally decompose in various medium in our environment. The depolymerisation results due to various physical and biological forces. The physical forces such as temperature, moisture, pressure etc, deals with causing mechanical damage to the polymer. The microbial biodegradation was widely accepted and still underway for its enhanced efficiency. Recently several microorganisms have been reported to produce polyster degrading enzymes.

Degradation of polymers in nature is well documented. It

constitute menace; where they constitute serious pollution, health hazard and endangerment to the environment. Removal of these pollutants by bioremediation using viable microorganisms is the crux of this research. The ability of Bacillus mycoides and Bacillus subtilis to biodegrade polyethylene was studied. Low density polyethylene and high density polyethylene films were exposed outdoor for 24 weeks. The two isolates were able to grow on polyethylene (PE) forming visible biofilms. The mean heterotrophic bacterial counts in the soil sample ranged between 2.311 X 10^5-3.20 X 10^8 CFU/g. The rate of degradation was determined by measurement of the residual weight of the PE films. Biodegradation in Erlenmeyer flasks by the bacteria after 60 days of incubation ranged between 8.41%-23.15%. The result showed that certain Bacillus sp. indigenous to the Niger Delta soil are capable of growing on PE films and biodegrade them, after an initial abiotic degradation. Bioremediation processes using the test bacteria is strongly recommended to be incorporated into waste disposal systems for a clean and healthy environment

Key words: Bacillus mycoides, Bacillus subtilis, biodegradation, natural weathering, waste disposal, polyethylene, plastics.

follows a sequence in which the polymer is first converted to its monomers, before they are mineralized. Most polymers like polyethylene are too large to pass through cellular membranes, so they must first be depolymerized to small monomers before they can be absorbed into the microbial cells. The degradation of most synthetic plastics in nature is a very slow process taking over a thousand years and involves the synergistic action of environmental factors and microorganisms (Cruz-Pinto et al., 1994; Nanda & Sahu, 2010). Some studies (Glass & Swift, 1989; Imam et al., 1992; Gu, 2003) have assessed the biodegradability of some polyethylene films by measuring changes in physical properties, amount of CO_2 evolved or by observation of microbial growth after exposure to biological or enzymatic environments.

Polyethylene (PE) littering of the environment is a common problem in most urban centers in Africa, because majority of PE wastes are often not recycled in most countries in Africa. This is because of the poor waste management practice in Africa. Within the last few decades there has been an increasing rise in plastic waste entering into the municipal solid waste streams in large cities in subSahara Africa.

These plastic wastes are causing an increasing number of environmental and health problems to humans and other animals. It has reportedly caused over a million deaths of marine animals (Barnes et al., 2009). These wastes have virtually choked the drainage system in the urban centers of countries in this region, to such an extent that it takes only the slightest of rainfall to precipitate floods in major cities in these areas, as is presently witnessed in Asaba, Onitsha and other cities.

Polythene and Plastic wastes range in types including pure water sachets, cellophane bags, plastic packaging of confectioneries, bakery products, toiletries, laminating materials, soft drinks, pieces of plastic buckets, plates, cups, wrappings etc. They are found in household wastes, refuse dump sites, municipal drainage system, street refuse collections, where they constitute menace; where they constitute serious pollution, health hazard and endangerment to the environment.

Vast amount of solid wastes are produced that require safe disposal. Urban solid waste production in Nigeria is estimated to cost several millions of Naira per annum (FEPA, 2010). Part of the material is inert,

composed of glass, metals, plastics, etc, but the rest is decomposable solid organic waste.

In traditional small-scale farm operations, most organic solid waste is composted and recycled to the land as fertilizers. In societies characterized by urbanization and large-s ale agriculture, the disposal of organic waste becomes a difficult and expensive problem (Bitten 1994).

The simplest way to handle solid waste disposal at the lowest direct cost is landfills. In this procedure, solid wastes both organic and inorganic are deposited in low-lying and hence low value land. Within the landfill, anaerobic and facultatively anaerobic microorganisms attack the organic compounds in the waste (Senior, 2005).

Composting of organic waste appears to offer an attractive alternative to landfills for the decomposition of solid domestic and agricultural wastes (Diaz et al 1993). Compared to alternative disposal methods, composting has considerable environmental advantages. Composting requires some initial sorting of the solid waste into organic and inorganic portions as only the organics will decompose. This can be accomplished either at the source by separate collection of carbage and trash, or at the receiving facility.

Plastics are essentially a modern group of materials, having only been commercially in use in any quantity for fifty years (Colin et al., 1981). The generic term plastics covers a wide range of diverse structures, and indeed does not limit itself to the polymer, but includes formulations, composites and copolymers (Klausmeier, 1996). Thus when considering susceptibility to attack, it is important to know exactly what the plastic represents in terms of its overall composition. Plastics may contain a wide variety of additives (Table 1), and may have residual processing aids adhering to their surfaces; all of these substances affect their susceptibility to microbial attack (Allsopp and Seal, 1986).

Table 1: Some additives used in synthesis of plastics

Pigments
Antimicrobial agents
Metals
Polymer flow controllers

Releasing agents
Lubricants
Plasticizers
Ultraviolet absorbers
Hydrolysis stabilizers

Studies have shown that among the various additives, the plasticizers based on adipates and sebacates are generally susceptible to biodegradation resulting in loss of plasticity of the microbiologically inert polymer (polyvinyl chloride, PVC). Other types of plasticizers are resistant when present as sole source of carbon, but the presence of the other organic nutrients in the medium stimulates their utilization. Whether this is a cometabolic or an induced enzyme process is not yet known (Pathirana and Seal, 2004). Plasticizers are esters which are broken down by the esterases found throughout the fungal and bacterial orders (Williams and Dale, 2003.)

Table 2: Examples of biodegradable commercial plasticizers

Adipates	*Sebacates*
Dihexyl adipate	Dimethyl sebacate
Dibutoxyethyl adipate	Dibutyl sebacate
Dicapryl adipate	Doctyl sebacate
Diiso-octyl adipate	Polypropylene sebacate
Dicotyl adipate	Dibenzonyl sebacate

Some of the earliest commercially produced plastics were regenerated proteins. These polymers have been shown to be subject to hydrolytic attack at the carbonyl sites (William and Dele, 2006), and their susceptibility to microbial attack supports this observation. Urea formaldehyde is an example of this class of polymers, which is limited in its use to surface coatings, and as a composite with compressed paper in domestic worktop laminates. Studies have shown that the properties of the polymers in this class such as their brittleness and hydrolysis in contact with water enhance their biodegradability.

Materials and Methods

Sample collection and source of Polyethylene: The soil was collected from the farmland located in Federal College of Education (Tech), Asaba Delta State. The sample was excavated with a trowel at 5cm depth and collected in a plastic crate. Preliminary weathering of polyethylene: Pre-weighed LDPE and HDPE films were exposed to sunlight on the roof of School of Science Laboratory Complex for 3 months. Exposure was performed according to ASTM standards, using 45°-angle wooden racks, facing south (Yabannavar & Bartha, 1994).

Isolation, enumeration and identification of polyethylene degrading fungi: Isolation and enumeration: Soil samples were collected from the upper 0-5 cm layer of the soil, stored in plastic crates and transported to Laboratory where it was kept at room temperature. The cut PE films were then buried in the soil after liming with Ca_2CO_3 and fertilizer $(NH4)_2HPO4$ application. After 1, 2 and 3 months of burial, the PE pieces were removed and transferred onto nutrient agar (NA) plates and incubated at 30°C for 24 hours, for bacterial isolation from the surface of PE. The direct isolation process was carried out by adding 1g of soil to 9 ml of sterile distilled water in a test tube to yield a 10-fold dilution. Next, a series of 10-fold dilutions were made in which 1 ml of each dilution was cultured on NA. Pure cultures were finally obtained by selecting a single colony of growth from highly diluted cultures. After incubation, the plates showing the most growth were chosen for enumeration.

Identification of polyethylene degrading bacteria: The identification of the bacterial isolates with the ability to degrade PE was performed on the basis of macroscopic and microscopy examination and biochemical tests. The bacterial isolates were identified macroscopically by examining colony morphology; surface pigment, size, margin and microscopic examination including Gram staining and motility test. The biochemical tests conducted were sugar fermentation, nitrate reduction, oxidase, citrate and catalase tests. The bacterial isolates were identified based on the keys detailed by Aneja (2003). Further identification was carried out using API 50 CHB analytical kit.

Fourier transform infrared spectroscopy (FTIR) was used to confirm biodegradation by determining the formation of new functional groups or disappearance of groups in the polymer ((Milstein et al., 1994).

Changes in the polyethylene structure following natural weathering and subsequent incubation with the fungal isolates were analyzed by FTIR spectroscopy (Perkin Elmer Spectrum BX11). The carbonyl index was measured from the FTIR spectrum in the transmittance mode, by comparing the relative intensities of the carbonyl band at approximately 1715 cm-1to that of the methylene band at approximately 1465 cm-1.

Results

Isolation and identification of microorganisms capable of degrading polyethylene: Table 3 shows the mean heterotrophic bacterial load in the soil. The mean heterotrophic bacterial count ranged from 2.81×10^5 - 3.20×10^8. The microbial count increased steadily with increase in time of burial. There were more microorganisms attached to LDPE films than to HDPE. Among the isolates screened for ability to degrade the LDPE and HDPE films, two bacterial isolates exhibited the fastest growth. The isolates were identified as *Bacillus mycoides* and *B. subtilis*.

Table 3: The mean heterotrophic bacterial count in the soil

EXPOSURE TIME (months)	HDPE	LDPE
0	2.41×10^4	2.41×10^4
1	2.50×10^5	3.22×10^5
2	1.50×10^6	2.54×10^6
3	2.29×10^7	3.71×10^7

Table 4: Morphological and biochemical characteristics of *Bacillus mycoides* and *Bacillus subtilis*

Charateristics	Bacillus mycoides	Bacillus subtilis
Morphology		
Straight rod	+	+
Gram's Reaction	+	+
Cell arrangement	Short chain, single	Short chain, single
Spore	Central	Central
Motility	-	+
Sugar fermentation		
Glucose	+	+

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Lactose	-	+
Sucrose	+	+
Fructose	+	+
Mannose	+	+
Sorbitol	-	+
Oxidase	-	+
Catalase	+	+
Citrate	+	+
Nitrate reduction	+	+

+ = positive; - = negative

Residual weight measurement for films incubated with microbial isolates: The results of residual weight measurement of pre-exposed and unexposed LDPE and HDPE applied to microbial treatment by incubating with individual isolates and microbial consortium in mineral salt liquid medium, expressed as percentage loss in weight, are given in Table 5 & 6.

Table 5: percentage biodegradation of PE films by isolates after 45 days of incubation at room temperature

TREATMENT	% WEIGHT LOSS (HDPE)	% WEIGHT LOSS (LDPE)
Pre-exposed film + Bacillus mycoides	7.31 ± 0.12	9.30 ± 0.40
Pre-exposed film + Bacillus subtilis	13.50 ± 0.40	22.10 ± 0.32

Table 6: Percentage biodegradation of PE films by isolates after 60 days incubation at room temperature

TREATMENT	% WEIGHT LOSS (HDPE)	% WEIGHT LOSS (LDPE)
Pre-exposed film + Bacillus mycoides	10.50 ± 0.17	11.35 ± 0.05
Pre-exposed film + Bacillus subtilis	17.72 ± 0.10	23.15 ± 0.05

Discussion

The results revealed that the heterotrophic bacteria count ranged from 2.81 x 105 – 3.20 x 108 CFU/g. The total heterotrophic bacteria (THB)

count was found to agree with the THB from Oron mangrove sediment in Nigeria (Ekpo & Madu, 2005). The high count of the heterotrophic bacteria in the mangrove soil is considered advantageous because they contribute significantly to the microbial decomposition process in the soil. Results indicated an increase in count after three months of burial. Orhan et al. (2004) reported that an increase of bacterial population correlated with the signs of disintegration of mechanical properties of natural polymer films, indicating the role of biotic component in degradation process.

This result is similar to the observations of Kathiresan, (2003) who investigated the degradation of plastics in mangrove soil and observed that significant biodegradation occurred only after colonization of the plastic, a parameter that was dependent on the resident microbial populations. Bacillus sp have been isolated from the soil in the Niger Delta with some associated with the degradation of hydrocarbons associated with crude oil (Antai, 1990; Akpan-Idiok & Solomon, 2012; Eziuzor & Okpokwasili 2009). There is well documented evidence of indigenous Niger Delta soil microbes degrading the oil pollutants entering the terrestrial and aquatic ecosystem (Benka-Coker & Olamagin, 1995; Odokuma & Dickson 2003).

The same microorganisms that mediate the degradation of hydrocarbon are expected to degrade polyethylene since their degradation is similar (Arkatkar et al., 2009). Both *Bacillus mycoides* and *Bacillus subtilis* exhibited varying degrees of ability to biodegrade PE and it was assumed that their isolation from soil constantly polluted by oil spill could confer such degradative ability on them. Biodegradation in Erlenmeyer's flask showed no growth of the bacterial isolates on the untreated films. However, growth was observed on treated films serving as sole carbon sources, resulting in weight loss of the films. Result of residual weight measurement revealed a dry weight loss ranging from 10.4% - and 23.15 % for LDPE and 8.41% - 17.72% for HDPE at the end of the incubation period for treated films probably because of the presence of carbonyl groups while the untreated films remained unchanged.

A similar result was obtained by Sudhakar et al. (2007) when Bacillus species isolated from the marine environment was used to degrade thermally treated LDPE and HDPE (19% and 9% respectively). 2004

reported 11% reduction in gravimetric weight of polyethylene after treatment with Brevibacillus borstelensis and Kathiresan (2003) reported 20.54% by Pseudomonas species. These low rates of degradation are in agreement with the argument of Otake et al. (1995) that 10 years is a relatively short period for the biodegradation of synthetic polymers such as polyethylene. It has been severally reported that bacterial flora growing in a stressed environment usually harbor different types and numbers of plasmid (Baya et al., 1986).

Conclusion & Recommendations

The study revealed that initial abiotic treatment of the PE films ensured initiation of degradation. Abiotic pre-treatment lead to introduction of oxygen in the polymer matrix, to form oxygen containing compounds which were made available for utilization by the bacteria. *Bacillus mycoides* and *Bacillus subtilis* grew better on abiotically weathered PE films than on un-pretreated PE films. On the basis of this study it can be concluded that *Bacillus mycoides* and *Bacillus subtilis* indigenous to the Niger Delta mangrove soil have potential for use in biodegradation of PE. It is recommended that bioremediation systems be adopted in the management of public wastes particularly plastics and polyethylenes. Such systems should include *Bacillus* sp as biodegradation agents under optimum conditions.

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