ISOLATION OF MICROORGANISMS ASSOCIATED WITH DEGRADATION OF PLASTIC AND POLYTHENE IN SOIL

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ABSTRACT

Biodegradation of polythene bags (PB) and plastic cups (PC) in soil were studied for five weeks using the weight loss method. The results revealed that weight loss begins after the third week, the rate of biodegradation was observed to be minimal and ranged between 2.1 - 2.4% for PB and 0.17 – 1.2% for PC. Heterotrophic bacterial count ranged between $2.3X10^4$ – 6.8 X 10^4 cfu/g for PB and 2.3X104 – 5.4 X 104 cfu/g for PC, while fungal count revealed 2.2 x 10^4 – 8.9 x 10^3 cfu/g for PB and 2.3 X 103 – 8.3 X 103 cfu/g for PC. The following microorganisms were found to be associated with biodegradation of PB and PC in soil: *Streptococcus* species, *Staphylococcus* species, *Pseudomonas* species, *Micrococcus* species and *Aspergillus* species. *Micrococcus species* recorded the highest incidence for both PB and PC amongst the bacterial isolates whereas the fungus, *Aspergillus niger* occurred frequently for both PC and PB. The results revealed the role of microorganisms in the biodegradation of both PB and PC although at a slow rate.

Key words: Biodegradation, Plastic cup, Polythene bags, Soil

INTRODUCTION

Plastic and polythene are some of the most commonly found pollutants in most developing cities of the world. Their presence poses a great challenge and causes a lot environmental contamination on the land. Plastic is any material made up of large, organic (carbon-containing) molecules that can be formed into a variety of products. The molecules that compose plastics are long carbon chains (polymers) that give plastics many of their useful properties (Microsoft ® Encarta ® 2009). Polythene is the most widely used plastic; it is a thermoplastic polymer consisting of long chains of Ethylene. Disposable goods and packaging materials makes about one third of the total plastic production and hence have the largest environmental impact since the material used is biologically inert (Lee and levy, 1991). Microorganisms ranging from bacteria and fungi found in natural waters and soils have between them a very broad ability to degrade virtually all naturally occurring compounds as their source of carbon and energy (Atlas and Bertha, 1993). But the presence of many pollutants that microorganisms do not encounter initially raises concern over their ability to carry out biodegradation (Clarke, 1980). But the reason is that most of these new compounds do not have compatibility with its natural counterpart or were made from chemicals that either degrade slowly or are non biodegradable. Biodegradation of plastic and polythene is attracting attention in recent time because of the menace caused by the products. Although biodegradable polymers are now synthesized to replace or augment various plastics that were recalcitrant, many microorganisms are capable of degrading most

of these compounds. This study was carried out to isolate and characterize the various microorganisms associated with the biodegradation of polythene bags and plastic cups that were on sale within Kaduna Metropolis and to evaluate the effectiveness of their activity.

MATERIALS AND METHODS

Sample Collection and Preparation: Fifteen samples each of plastic cups and polythene bags were purchased from Abubakar Gumi central market Kaduna. Each sample was weighed and then buried at a depth of 5cm in the soil of the biological Sciences garden of Kaduna State University, Kaduna. The samples were allowed to degrade naturally and then sampled again on a weekly interval for a period of 5 weeks using a sterile forceps and transferred to the laboratory for analysis in each case. Both the polythene and plastic cups were washed with distilled water, dried in the shade for 12 hours and then weighed. The soil sample from where the samples were buried was also collected in a sterile polythene bag and transported to laboratory for analysis.

Enumeration of Microorganisms: One gram of the soil sample was weighed and dissolved in 9ml of distilled water in a sterile test tube. The dilutions ranged from 10^{-1} to 10^{-10} according to the method of Collins and Lyme (1979). Appropriate dilutions of samples were inoculated on Nutrient Agar (NA) and potato dextrose agar (PDA) by pour plate techniques for the enumeration of bacteria and fungi respectively. The NA plates were incubated in an incubator at 37^{O}C for 24-48 hours while the PDA plates were incubated at room temperature for 3-5 days.

Identification of Bacteria: Pure cultures of bacterial isolates were obtained from the primary culture after sub culturing using NA plates which were incubated overnight at 37^{O}C. Upon sub culturing, bacteria that grow as discrete colonies were stored on nutrient agar slants for further characterization and identification.
This was done first by examining the colonial morphology on growth medium and cellular morphology under a light microscope.

Gram's Staining: Colonies of the suspected isolates were Gram stained to label the organism as either Gram positive or Gram negative. Gram positive are those organisms able to retain the primary color of the crystal violet whereas Gram negative retain the red color of the counter stain (Safranin). Gram staining was done by first preparing a thin smear on a clean grease-free glass slide, a metal wire loop was used to transfer a small portion of the organism and a little drop of water was added and then emulsified on the glass slide. The smear was then air dried, fixed and stained with crystal violet and washed slowly with water, iodine solution was added for a minute and then washed with water. The smear was then decolorized with acetone (alcohol) and washed immediately with water, it was then flooded with safranin (counter stain) and allowed to stand for 30 seconds and then washed with water. The smear was air dried and then observed under the microscope.

Biochemical Tests: The following biochemical tests were carried out to identify the isolated organisms using the method described in Oyeleke and Manga (2003) and Cheesbrough (2006): motility test, indole test, urease test, catalase test, coagulase test, citrate utilization test, and triple sugar iron (TSI) test.

Motility Test

This was done to determine the presence or absence of flagella. Tubes containing the motility medium were inoculated by making a fine stab with a loopful of the culture to a depth of 1-2cm and were then incubated for 24-48hrs at 37^{o}C. If the bacteria are motile, the medium become cloudy.

Indole Test: This test was carried out to identify enterobacteria because of their ability to break down the amino acid tryptophan with the release of indole. The organism was grown in a bijou bottle containing 5ml peptone water for 24hrs after which 3 drops of kovac's reagent were added and shaken gently, red surface was examined within 10mins in the surface layer.

Urease Test: This test is important in differentiating enterobacteria. The organism was cultured in a urea agar slant in a test tube and incubated for 24-72hours. The presence of a bright pink and red colour was examined.

Catalase Test: Catalase Protects bacteria from toxic hydrogen peroxide **(H_2O_2)** accumulation, which can occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. The test was carried out by adding several drops of 3% H2O2 on a glass slide. A portion of growth of the organism was then picked from a slant with the aid of a wire loop and added to the slide. A positive test was indicated by bubbling and frothing which were absent in a negative test.

Coagulase Test: This was done to identify *Staphylococcus aureus*. In this test, two drops of physiological saline about 2cm apart were dropped separated by a mark on a glass slide. A colony of the organism was emulsified in each of the drops to make two thick suspensions; a loopful of little citrated human plasma was added to one of the bacterial suspension and mixed with the loop. The slide was held up and little back and forth for a minute and clumping of the cells was observed.

Citrate Utilisation Test: This test is based on the ability of an organism to use citrate as a carbon source. Slopes of the simmon citrate agar were prepared in a bijou bottle; a wire loop was sterilized and then used to pick the test organism which was streaked on the slope. It was then incubated for 24-72 hours. The presence of a bright blue colour was examined.

Triple Sugar Iron Test: This test is based on the fermentation of glucose and lactose, production of gas and the production of hydrogen sulphide. A sterilized straight wire loop

was used to stab the butt of the TSI medium with the test organism 2-3 times. The same wire loop was also used to streak the slant which was then capped loosely and incubated at 37oC. The result was interpreted and recorded.

Identification of Fungi: The fungi were characterized based on their appearance on the potato dextrose agar and their microscopic appearance. The PDA plates were examined and pure culture of each isolates was prepared using lactophenol cotton blue (LCB). A drop of the LCB was placed on a free clean glass slide using a bent disinfectant needle. a small mass of the mycelia growth was carefully picked with the aid of a pair of sterile dissecting needles and placed on a drop of LCB on a slide, covered with a cover slip. The slide was examined under the microscope, first with($\times$ 10 examine under oil immersion to detect the shape and kind of asexual spore, presence of special structure such as foot cell, sporangiophore or conidiophores and the characteristic spore head. (Domsch and Gams, 1970).

RESULT AND DISCUSSION

From the result obtained, biodegradation of both polythene and plastic begin after the 3rd week, this indicates their slow biodegradation rate (table 1). The result of bacterial count each of polythene and plastic was presented in table 2. The range was between 2.3 – 7.4 x 10^4 for polythene and 2.3 – 5.4 x 10^4 for plastic. A number of four different bacterial species were identified, Micrococcus and Streptococcus recorded the highest number of occurrence of 26.7% each while *Pseudomonas* and *Staphylococcus* records 23.3% each for polythene while for plastic, staphylococcus, micrococcus and pseudomonas each records 26.7% against the 20% recorded by streptococcus (table 5). This variance indicates the high presence of these organisms in the soil. High number of bacterial species was recorded in plastic than polythene. This may be due to the prevailing soil conditions such as soil moisture and heat around the materials it is also possible that the organisms have competent enzymes system that could degrade the polythene and plastic material as reported (Kathieseran, 2003). For fungi, various species of Aspergillus were identified table 4. Fungal count was found to be higher in polythene than plastic. A. *niger* records high number of occurrence of 23.3% and 30% respectively in polythene and plastic followed by A. flavus, 20% and 26.7%, A. fumigatus 20% and 23.3%, A. candidum 20% each and then A.clavatus that records16.7% in polythene and 0% in plastic (Table 4).

CONCLUSION AND RECOMMENDATIONS

It can be concluded from this study that both Gram positive and Gram negative bacteria and fungi were involved in the biodegradation on both polythene and plastic but the biodegradation is very slow proving the recalcitrant nature of these materials, the biodegradation commences after two weeks. Based on the result it can be recommended that the manufacturers of these materials should use biodegradable environments. Members of the public should practice safe disposal of these materials.

Table 1. Biodegradation (%weight loss in soil) of plastic and polythene for three samples

Period	Polythene			Plastic		
Sample:	A	B	C	A	B	C
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0.8	0.8	1.6	0.10	0.2	0.07
4	1.2	1.0	1.8	0.21	0.8	0.07
5	2.4	2.1	2.4	0.65	1.2	0.17

Table 2. The bacterial count of polythene and plastic

Period (weeks)	Polythene (10^4)	Plastic (10^4)
0	2.9-4.0	3.6 - 4.2
1	3.2-5.4	2.3 - 4.4
2	4.2-6.8	3.9 - 5.3
3	3.6 - 4.2	2.9 - 4.0
4	4.2 - 7.4	3.0 - 5.1
5	2.3 – 4.4	3.2 – 5.4
Range	2.3 - 7.4	2.3 – 5.4

Table 3. The result of Fungal count each of polythene and plastic

Period	Polythene ($\times 10^4$)	Plastic ($\times 10^4$)
0	3.2 – 5.2	6.5 – 7.2
1	2.2 – 4.6	4.0 – 5.3
2	6.5 – 7.2	3.2 – 5.2
3	5.8 – 8.9	4.6 – 8.2
4	3.9 – 7.4	3.0 – 5.3
5	3.2 – 4.5	2.3 – 4.4
Range	2.2 – 8.9	2.3 – 8.2

Table 4. The percentage of Bacterial species Isolated

Organism	Polythene (%)	Plastic (%)
Staphylococcus sp	23.3	26.7
Streptococcus sp	26.7	20.0
Micrococcus sp	26.7	26.7
Pseudomonas sp	23.3	26.7
Total	100	100

Table 5. The percentage of isolated fungal species

Organism	Polythene	Plastic
Aspergillus niger	23.3	30.0
A. flavus	20.0	26.7
A. fumigatus	20.0	23.3
A. candidum	20.0	20.0
A. clavatus	16.7	0
Total	100	100

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