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BIODEGRADATION OF POLYTHENE BAGS BY SOME SELECTED BACTERIA AND IDENTIFICATION AND CHARACTERIZATION OF POLYTHENE DEGRADING MICROORGANISM

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ABSTRACT
Polythene waste accumulating in the environment poses an ever increasing ecological threat. They are usually polymer ethylene which forms an essential part in our daily life. They are used in various sectors of wide application such as packaging materials as films or sheets. In this study biodegradation of polythene bags by some selected Bacteria and identification and characterization of polythene degrading micro-organism was carried out. The polythene bags were buried on the ground for the period of nine months. Subsequently, the different samples of the same polythenes were inoculated with Bacillus subtilis and Staphylococcus aureus for one month with addition of 15ml fresh media to the medium every week. Serial dilution of 10-1 – 10-4 dilution was carried and pour plate method was done to obtain a discrete colony for both the buried and Laboratory inoculation samples. The isolates were

Introduction:
The disposals of domestic, industrial and urban wastes have constituted a lot of environmental pollution. These human activities had led to water and soil contamination which invariable had affected directly or indirectly the health of both human and animals. The major challenge is the non-biodegradability or slow rate of degradation of some of the pollutant under natural condition (Sangale et al., 2012). Polyethylene constitutes a large portion in most of our environmental wastes due to it great demand in sealing, bagging wrapping and packaging of our goods. Polyethylene is a polymer of ethylene gas (CH2 = CH2) having the chemical formula (C2H4) nH2. There are

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identified by gram stain reaction and biochemical test. In coagulase and catalase test, 10-1 – 10-4 dilution were positive. The fungal species associated with the polythene bags that were buried were Cladosporium species, Aspergillus species, Rhizopus Species, Candida Species, Mould and Fusarium species. The bacterial isolate are Staphylococcus species, Streptococcus species, Pseudomonas species, Bacillus species. The percentage weight loss of polythene bags inoculated with Bacillus subtilis and Staphylococcus aureus , for one month labeled A,B,C,D and E were(33.33, 29.35, 33.72, 25.00 and 40.00; 23.72, 35.48, 49.01, 41.66 and 25.00%) respectively. While the percentage for the buried samples were (28.75, 19.89, 24.94, 23.35 and 15.66%). This studied showed that some microbes are capable to some extend degrade polythene. In comparison there is greater percentage weight loss in Laboratory condition than the natural environment

***Keyword**: Polythene, Staphylococcus aureus, Biodegradation and Fusarium species, Bacillus subtilis*

Approaches were made to reduce the nuisance this polymer has created in the entire globe. Some of these approaches were; thermal treatment, land filling, recycling and biodegradation. In a recent time the focus has shifted to biodegradation using various microorganisms and value had been recovered from polythene (David et al., 2009). Various categories of polyethylene which include low density (VLDPE) (Rivard et al., 2005). Polyethylene is one the most abundant commercially produced synthetic polymer and there is an increasing demand of these materials in transportation, food clothing, shelter construction medical and recreation industries (Adeline et al., 2009). Among the non-biodegradable polymers, polythene is mostly used in human's daily because of its easy processing for various products (Arutchelvi et al., 2008). Polythene is known to be a global threat to human, land and aquatic habitats. On land, it causes the death of some animal due to the blockage of their digestive tract as a result of undigested polythene (Manisha et al., 201 2; Singh, 2005). In aquatic environment, polythene is

also known to cause internal blockage of the fishes and other aquatic animals (Francis et al., 2010). The bio-degradation of polyethylene can occur by different molecular mechanisms such as chemical, thermal, photo and bio-degradation. Bio-degradation is evaluated by weight loss, tensile strength loss, changes in percent elongation and changes in polyethylene molecular weight distribution physiochemical distribution is initiated by treatment with acid at 700C and irradiation of the polyethylene film (Shah, et al., 2008). Biodegradation is defined as the breaking down of organic materials by microorganism leading to mineralization of the environment. However, in most cases the term biodegradation is generally used to describe any biologically mediated changes in a substrate (Aswale, 201 0). So understanding the process of bio-degradation requires transform the substances through metabolic enzymatic processes. It is based on two processes: growth and cometabolism (Viswanath et al., 2008). Cometabolism is defined as the primary carbon and energy source. Several micro-organisms, including fungi, bacteria and yeasts are involved in bio-degradation (Viswanath et al; 2008). Bio-degradation processes vary greatly, but frequently the final product of the degradation is carbon-dioxide. Organic material can be degraded aerobically, with oxygen or an aerobically, without oxygen. Biodegradable matter is generally organic material such as plant and animal mater and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by micro-organism. (Albertsson, and Banchidi, 2005). There is no such report inn case polythene degradation but a similar trend is predicted. The byproducts of the polythene varied depending upon the conditions (Adetuyi, 2010). Carbondioxide water and microbial biomass are the final degradation products where as in case of anaerobic methanogenic condition carbondioxide water, methane and under sulfidogenic condition H2S, carbondioxide and water and microbial biomass are reported to the end product (Cornell et al., 2005). Polyethylene is very resistant to bio-degradation due to its high hydrophobicity and its long carbon chains (Conta and Ribes 2007). Under normal condition, it takes more than 10 decades to mineralize the polymers (Ohtake et al., 2010). Several cities in

Nigeria have prohibited distribution of plastic bags by supermarkets and other commercial establishments because they are made of various chemicals which are toxic to the health and the environment. An estimated one million birds and ten thousands marine animals die each years as a result of ingestion of plastics (Adetuyi, 2010). Municipalities have failed to manage polythene waste probably due to financial factors and altitude of our people to land use management and environmental issues. Recycling makes a nation or individual to recover money otherwise lost through pollution, burning and burying of waste materials. This polyethylene waste recycling will complement the international concern for the environment and our government's campaign against disforestation and ozone layer depletion which leads to climate change. It will reduce environmental pollution caused by polyethylene materials littering the street, bus stops, schools market and blocking our drainages used polyethylene bags and plastic materials productivities of such lands. Removing these materials from the farms for recycling will create job opportunities improves soil structure increase crop yield potential of the land and consequently contributes to national food security. Due to the negative impact of polythene to the environment, different

In biodegradation, strong carbon bonds are broken down through microbial actions that reduces the strength of polythene (as molecular weight decreases) and hence polythene gets degraded (Pruter, 2000). Polyethylene can be degraded through two ways aerobic as well as anaerobic. In aerobic degradation oxygen acts as an electron acceptor and final products are carbon dioxide and water (Seymour 2010). Anaerobic biodegradation occurs in absence of oxygen and therefore microorganisms use nitrate, sulphate and iron as electron acceptor Aswale (2010) tested the toxicity level of all the polythene biodegraded products in both the animal and plant systems. It was found that in plant systems, toxicity of the degraded polythene products with culture filtrate moderately decreased the seed germination rate of the Arachishypogaea (groundnut) Glycine max (soy beans), sesamumlaciniatum (oil seed, sesame), Helianthus annuus (sunflower) and carthamustinctorius (safflower). For the animal system, the mortality rate of chironomous/arvae was calculated, and found

no significant different in the mortality rates as compare to control (Siddiquee et al., 2014). The polythene and plastic degradation site in the soil can serve as a dumping ground for these materials. It was found that polythene biodegradation is relatively faster than that of plastics. Priyanka et al., 2011 reported their finding after the isolation of 5 species of bacterial and fungal Pseudomonas spp Bacillus subtilis, Staphylococcus aurecus, Streptococcus lactis, Proteus vulgaris, Aspergrittus flaucis, Aspergillus glaucus, Pencillium SPP. These microbial species were tested in the laboratory for their ability to degrade the polythene and plastics. It was found that their degradation ability was faster in the laboratory condition. This research is aimed at investigating the biodegradation of polythene bags by some selected organisms.

METHODOLOGY

PREPARATION OF CULTURE MEDIA

Preparation of nutrient broth

About 4.9g of the medium was weighed accurately using weighing balance and was poured in a sterile 500ml conical flask, 375ml of distilled water was added and covered with cotton wool and sealed with aluminium foil paper and sterilized in a bucket autoclave of 121 oC for 15 minutes. These preparation was made in 3 different 500ml conical flask getting a total medium preparation of 1,500ml of the nutrient broth.

Preparation of nutrient agar

About 10.5g of the nutrient agar was weighed accurately using weighing balance and was poured in a sterile 500ml conical flask, 375ml of distilled water was added to it using measuring cylinder, it was then covered with cotton wool and sealed with aluminium foil paper and sterilized in an autoclave of 121 oC for 15 minutes.

Preparation of sabouraud dextrose agar

About 24.4g of the medium was weighed using weighing balance and was poured in a sterile 500ml conical flask, 375ml of distilled water was added to it using measuring cylinder, it was then covered with cotton wool and sealed with aluminium foil paper and sterilized in an autoclave at 121 oC for 15 minutes.

MICROBIAL DEGREDATION OF POLYTHENE IN LABORATORY CONDITION

Determination of weight loss using Bacillus subtilis and Staphlococcus aureus

The samples were labeled A-E in duplicate and weighed severally until a constant weight was achieved, Ten (10) conical flask was set and 500ml of nutrient broth was measured into each of the flask, five (5) flasks was incubated with Bacillus subtilis and the other five (5) with Staphlococcus aureus. The first set of the pre-weighed pieces of sample were aseptically transferred into the flasks containing of Bacillus subtilis and the second set of the preweighed pieces of the sample were also transferred aseptically into the flask containing the Staphlococcus aureus bacteria. This set-up was maintained for one month and weekly addition of the broth with 15ml fresh nutrient broth was done to enhance the growth of be inoculated bacteria for effective degradation. After one month of this experiment the polythene piece were then removed and washed through using distilled water, it was air-dried and then weighed for final weight. The percentage (%) weight loss was obtained by the formular below.

$$\text{Percentage weight loss} = \frac{At - Ai}{At} \times 100$$

Where At = Initial weight of the sample
Ai = Final weight of the sample

Microbial degradation of polythene under natural condition

The second set of the samples were buried in the soiled of about 12 inches deep. The hole was sealed with the soil and the site was demarcated and was constantly wet with water to achieved a moist soil for effective degradation. These set-up was made for a period of 9 months, after it was unburied and picked with a sterile forceps into a sterile conical flask and was sealed with cotton wool. The samples were taken to the laboratory washed with sterile water and the solution was use for identification and characterization of micro-organisms and to check the percentage weight lost.

ISOLATION AND IDENTIFICATION OF THE MICRO – ORGANISM

Serial dilution

The buried samples were washed vigorously with sterile distilled water to dislodge. Serial dilution tubes was labeled from 10-1 to 10-4, 1ml was transferred into 10-1 from the stock using a sterile pipette, 1ml from 10-1 to 10-2 1ml from 10-2 to 10-3, 1ml from 10-3 to 10-4 respectively, for each of the dilution tube 0.1ml of dilution slant was transferred to nutrient agar plate and was incubated at 35oC to achieved growth.

IDENTIFICATION OF THE BACTERIA

Gram staining and biochemical test such as coagulase test, catalase test were performed to observe the cellular microbiological and gram nature of the isolates.

Results

Table 1: Total heterotrophic count

S/N	Dilution factor	No of colonies	Inoculums	Cfu/ml
1	10 -1	129	0.1ml	129
2	10-2	62	0.1ml	6.2
3	10-3	46	0.1ml	0.46
4	10-4	22	0.1ml	0.022

Table 2: The rate of degradation of polythene bags by Bacillus subtilis

S/N	Polythene bags	Initial weight (g)	Final weight (g)	Differences	Weight loss%	Period of incubation
1	A	0.51	0.34	0.17	33.33	1month
2	B	0.31	0.25	0.10	29.35	1month`
3	C	0.51	0.44	0.07	33.72	1month
4	D	0.12	0.09	0.03	25.00	1month
5	E	0.50	0.30	0.2	40.00	1month

Table 3: Rate of degradation of polythene bags by Staphylococcus aureus

S/N	Polythene bags	Initial weight (g)	Final weight (g)	Differences weight(g)	Weight loss%	Periodo incubation
1	A	0.51	0.44	0.07	23.72	1month
2	B	0.31	0.20	0.11	35.48	1month`
3	C	0.51	0.26	0.25	49.01	1month
4	D	0.12	0.07	0.05	41.66	1month
5	E	0.50	0.40	0.01	25.00	1month

Table 4: Difference weight loss of polythene bags samples buried in the ground

S/N	Polythene bags	Initial weight (g)	Final weight (g)	Differences	Weight loss (%)	Weigh Period of Incubation
1	A	6.99	4.98	2.01	28.75	9month
2	B	3.72	2.98	0.74	19.89	9month`
3	C	4.85	2.67	2.18	24.94	9month
4	D	1.67	1.28	0.39	23.35	9month
5	E	16.60	14.0	2.6	15.66	9month

DISCUSSION

From this finding, some microorganisms are capable of degrading polythene both in the natural and Laboratory conditions. These results are in agreement with (Rutkowska et al., 2002) which stated that the biodegradation is a natural process of degrading materials through microbes such as bacteria, fungi and algae. This is also in line with (David et al., 2009) reported that the isolates have degradation potential to degrade the polythene bags. Biodegradation on polythene bags was carried out by Bacillus subtilis and Staphylococcus aureus. The bacteria isolates shows the following distribution as represented in the tables;

Table 1 shows total heterotrophic count of the bacteria isolates with the dilution factor of 10-1 has the highest colonies (129Cfu/ml), the least was 10-4 with (0.022 Cfu/ml). (Albertsson et al., 2005)

Table 2 shows the percentage degradation of different polythene bag by Bacillus subtilis. It was observed that the highest degradation was seen in sample E with 40% for one month period of incubation.

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Table 3 shows the percentage degradation of polythene bag by Staphylococcus aureus in percentage, it was observed that the highest degradation was observed in sample C at 49.01% after one month period of incubations.

Table 4 shows the different in the weight of polythene bags samples buried on the ground. It was observed that the highest degradation was seen in sample C at 44.94% after nine month period of incubation on the ground. There is a significant different from the initial weigh of the sampled polythene bags with the final weight of the polythene. This indicate that the isolate have degradation potential to degrade the polythene bags.

CONCLUSION

In conclusion, due to addition of fresh nutrient to culture medium weekly, the isolates were being enriched and enhance their degradation ability unlike in the natural environment were the nutrients may be depleted after some time making the Laboratory condition more effective for biodegradation of polythene bags.The result shows that, the degradation rate is faster in the laboratory condition than in the soil. It was also shown that the result of Bacillus subtilis and Staphylococcus aureus in the degradation of polythene shows that Staphylococcus aureus has higher potential of degradation over Bacillus subtilis.

RECOMMENDTON

From this studied, it can be deduced that provision of Laboratory condition will enhance biodegradation of polythene, given that fresh media can be added to replenish depleted medium. Also additions of nutrients to the natural environment where polythene bags are collected for degradation will stimulate the potential of the microorganisms within that environment to degrade the polythene.

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