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Isolation of Bacteria from Landfill Sites in Kumasi Which Degrade Pre-Treated Plastic Bags

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ABSTRACT

Biodegradation of plastics could be a step in controlling plastic waste pollution. Bacteria were isolated from a landfill at Dompoase and another minor dump site at Bomso, all in Kumasi. Five bacterial strains were isolated and identified using biochemical tests and API 20E and 50 CH. The bacteria were used to degrade plastic bags previously treated with concentrated hydrochloric acid. The plastics were exposed to bacteria under aerobic and anaerobic conditions and a combination of the pure strains of the five isolates were used to degrade treated or untreated plastics. The subsequent loss in weight after the incubation period was used as a measure of percentage biodegradation. *Bacillus firmus* and *Proteus mirabilis* reduced the weight or degraded the plastics by 30.2% and 30.3% respectively under aerobic conditions whiles *Enterobacter cloacae* and *Proteus mirabilis* did by 11.9% and 11.7% respectively under anaerobic conditions. Statistical analysis indicated that the difference in percentage biodegradation was due to the different growth conditions i.e. aerobic or anaerobic (p – value 0.01 is significant). The combination of the pure bacterial strains degraded treated plastic bags to a larger extent than untreated plastic under both aerobic and anaerobic conditions i.e. the initial weight of treated plastic reduced by 9.58% in aerobic and 5.70% in anaerobic compared to 0.52% in aerobic and 2.13% in anaerobic of untreated plastic (p – value <0.001 significant). The difference in percentage biodegradation was due to the treatment conditions of the plastics. Bacteria possess the ability to degrade plastic bags and this is facilitated by the pre – treatment with acid.

KEY WORDS; Plastics, percentage biodegradation, landfill, anaerobic and aeorobic.

INTRODUCTION

Plastics are synthetic polymers of carbon, hydrogen and oxygen derived from petrochemicals and natural gas (Sharma and Sharma, 2004). They are generally light weight, resistant to many chemicals, impermeable and recyclable (Greif *et al*., 1998). Ethylene and propylene used in the manufacture of plastics are petroleum products (Htay and Oo, 2008). Other elements like chlorine, oxygen, fluorine are present in the backbone of some plastics eg: polyvinyl chloride contains chlorine (Greif *et al*., 1998).

Plastic waste is of particular interest in the fight to rid our cities of waste because it persists in the environment for a very long time. It has been estimated that only 0.5% of a plastic material would degrade in hundred years. Improper waste disposal contributes to the annual floods experienced in Accra, Kumasi and Takoradi through the clogging of drains (Fobil and Hogarh, 2006) responsible for water borne diseases like diarrhoea and cholera. Fishes and birds may swallow plastics dumped in the sea, get choked and die as a result of immobilization (Franeker *et al*., 2009).Chemicals used in the manufacture of plastics leach into the environment when buried in landfill sites for a long time. The chemicals are toxic to humans and other living things. Examples are bisphenol A and Di (2-ethylhexyl) phthalate (DEHP), endocrine disruptors known to cause breast cancer (Akkhavong *et al*., 2009).

Biodegradation simply refers to the use of microorganisms to break down complex structure of plastics and utilise the carbon substrates from the plastics for growth (Heimowska *et al*., 2001). In India, two species *Pseudomonassp.* and *Aspergillusglaucus* were isolated from mangrove soil which degraded plastics in a month by 20.54% and 28.8% respectively (Kathiresan, 2003). Sharma and Sharma, 2004 also assessed the effectiveness of *Pseudomonas stutzeri*for its ability to degrade low density polythene and polypropylene previously treated with concentrated nitric acid.

Kumasi metropolis generates a total of 1500 tons of solid waste daily, 80% of which is disposed of in the only landfill at Dompoase and two other dumping sites at Ohweim and Amanfrom at a cost of GH¢8, 049, 600 annually (Personal communication).Unfortunately the remaining 20% stays in the environment contributing to a myriad of environmental problems. Studies show that plastic materials make up a huge chunk of this waste, but their actual quantum cannot be estimated since solid wastes are not segmented into components. Our study aimed to isolate microorganisms from the landfill sites in Kumasi and use them to degrade plastic materials.

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METHODOLOGY

Soil samples (1g) were collected from two distinct areas rich in plastic wastes at three different depths 10 cm, 30 cm and 100 cm at the landfill sites (Dompoase and Bomso) for analysis. Soil samples were collected from different depths to isolate different bacteria in the study area. For each sample, 10% w/v was prepared and serially diluted10^{-1}to10^{-8}in test tubes containing sterilized water. Samples were pour plated on sterile agar, incubated for 24 hrs at 37°C and coliform forming units counted with a colony counter.

Table 1: Category of soil samples collected

	10 cm	30 cm	100 cm
1	A	D	G
2	B	E	H

Identification of Bacteria

Microorganisms were subcultured twice to obtain pure strains, examined morphologically and gram stained. Catalase and Oxidase tests were conducted on the isolates according to manufacturer's instruction (McFaddin, 2000).Analytical Profile Index (API) 20E and 50CH were then used to identify the isolates. API 20E – 18-24 hour identification of *Enterobacteriacae* and other non-fastidious gram negative bacteria while API 50 CH – Performance of carbohydrate metabolism tests (http://www.biomerieux-usa.com).

Biodegradation assay

A modified method of Kathiresan, 2003 was used to analyze the biodegradation of the plastics. Pieces of plastics were placed in a beaker of 100 ml Conc. HClfor 5 days without heating. The plastics were washed with sterilized distilled water and solar dried. The isolates alone or combined were inoculated into Bushnell Haas medium with the pre-treated /untreated plastics and then incubated for 30 days under both aerobic and anaerobic conditions. Samples were left on the shaker for a month after which dry weights were taken and percentage weight losses estimated. Microbial growth was analyzed using a spectrophotometer at 480 nm at a four day interval for 30 days

RESULTS

Five isolates were obtained. *Enterobacter cloacae, Bacillus firmus, Bacillus sp. Proteus mirabilis, Pseudomonas aeruginosa.* The first 3were isolated from the landfill at Dompoase while the rest were obtained from Bomso site. All five isolates gave negative results when oxidase test was done indicating that they lacked the oxidase enzyme. All isolates tested positive for the catalase reaction. The positive reaction is shown by the formation of bubbles upon addition of hydrogen peroxide. All the isolates when viewed under the microscope were rod shaped. Two were gram-positive and 3-negative.Different bacteria were obtained at different depths shown below;

Sample code		Identified organism
B18	-	*Pseudomonas aeruginosa*
B8	-	*Proteus mirabilis*
D17	-	*Enterobacter cloacae*
D28	-	*Bacillus spp*
D7	-	*Bacillus firmus*

No bacteria were isolated from G and H samples collected from the depth of 100 cm.

Percentage biodegradation

Weight differences before and after incubation were used to estimate the percentage weight loss after the thirty day incubation period.

Thus % biodegradation = $\frac{M1-M2}{M1} \times 100\%$;

Weight before biodegradation = M1

Weight after biodegradation = M2

At the end of the incubation period, *Bacillus firmus*, *Proteus mirabilis* and *Pseudomonas aeruginosa* recorded the highest biodegradation of 30.2, 30.3 and 28.3% respectively under aerobic conditions (Fig 1).

Under the aerobic conditions, *Proteus mirabilis* recorded the highest biodegradation of 30.3 whiles *Bacillus sp*. recorded the least valueof 17.0%. *Enterobacter cloacae* also recorded the highest percentage under the anaerobic conditions and *Bacillus sp.* recorded the least i.e. 11.99% and 0.098% respectively (Fig 1).

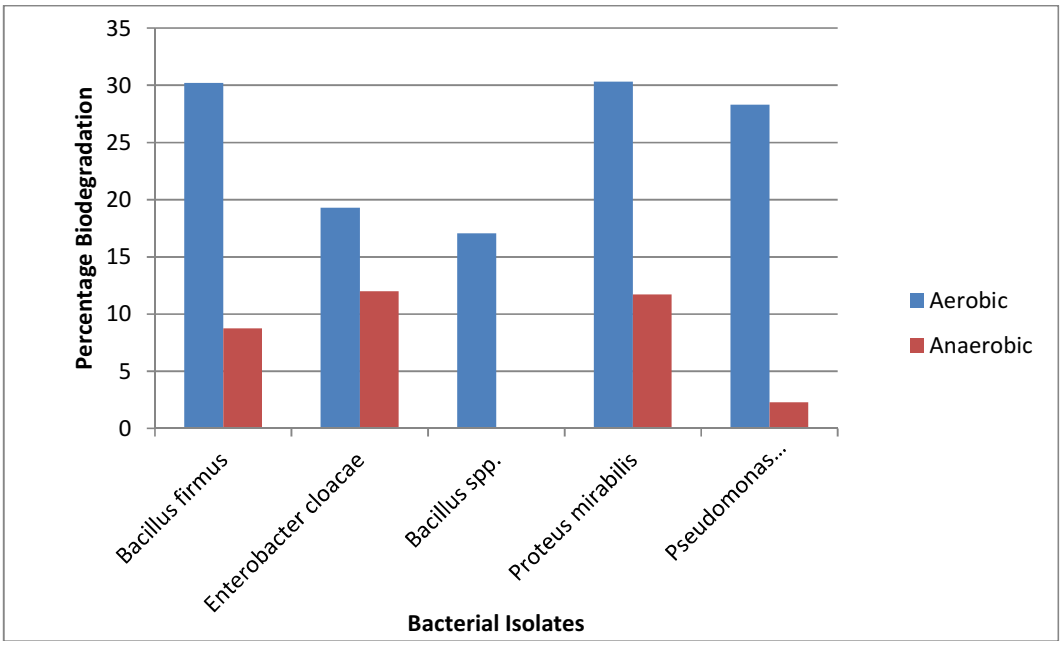

Fig 1: **Percentage biodegradation under aerobic and anaerobic conditions**

Percentage biodegradation of plastics under aerobic conditions was significantly different from that for anaerobic conditions; p – value = 0.01. It was observed that individual organisms degraded to a larger extent than the consortium of organisms (Fig 2).

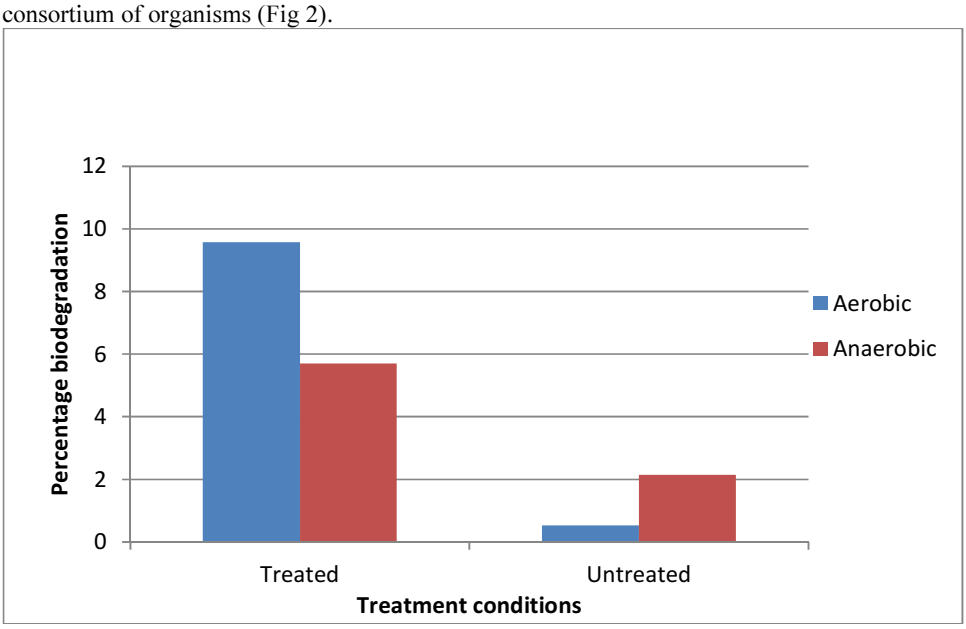

Fig 2: **Percentage biodegradation of treated and untreated plastic using all five isolates.**

The treated plastic showed a higher percentage biodegradation9.58under the aerobic incubation compared to 0.53% for untreated plastic. The value reduced to 5.70 and 2.31% respectively (Fig 2).

DISCUSSION

Five isolates were identified and used for biodegradation of the plastics. This number is significantly lower than the 43 isolates obtained by Christi *et al.,* (2007).In their case samples were collected from the mangrove

soil rich in heterotrophic bacteria as against our collection from a landfill and dump site. The 5 isolates are close to 7 obtained by Kathiresan (2003) from already degrading polythene buried in the mangrove soil.

Pseudomonas sp. was found to degrade plastics by 20.54% (Kathiresan, 2003). In this study, *Bacillus firmus* and *Proteus mirabilis* caused a higher weight reduction by 30.2 and 30.3% (Fig. 1) respectively under aerobic conditions. This may be attributed to the differences in organisms involved in the degradation irrespective of the pre – treatment procedure. However *Pseudomonas aeruginosa* also showed a higher weight loss of the plastic analysed by 28.3% (Fig. 1). The difference in the degradation of the plastic materials may be due to oxidising agents (different acid) used in the treatment. Oxidising agents started the cleavage of the extensive polymer chain of the plastic materials (Bonilla and Lobo, 2003).

Organisms were isolated from the landfill where they lived in association with each other. However the combination of pure bacterial strains in the degradation process yielded considerably lower results as compared to the individual strains (Fig. 1 and 2). This may have resulted from the inability of the isolated organisms to form mutual relationships with each other due to competition for the same carbon substrate.

Pseudomonas aeruginosa and *Bacillus sp.* showed no growth activity during the incubation. However at the end of the incubation weight losses of 2.30 and 0.09% respectively were observed which contradicted the result obtained for growth (Fig. 1). This may be due to the pre – treatment with acid which without the action of the bacteria started the cleavage of polymer chains of the plastic and the eventual weight loss.

Analysis of variance of the percentage biodegradation under different growth conditions: aerobic and anaerobic, showed that the conditions had a significant effect on the biodegradation (Fig. 1). This phenomenon explains the relatively slow rate of biodegradation in landfills; the landfill environment is said to be anaerobic. Results showed that prior treatment of plastics recorded a considerably higher increase in percentage biodegradation indicating that the acid played a part in breaking down the complex polymer chains of the plastic into smaller monomers before digestion by bacteria.

Conclusion

Five bacterial strains were isolated; they were *Proteus mirabilis, Enterobacter cloacae, Bacillus sp*., *Bacillus firmus*and *Pseudomonas aeruginosa.* The isolates degraded the plastic to a higher extent when used individually and under aerobic conditions. Treatment with a strong oxidising agent (HCl) startedthe process of breaking down polymer chains of the plastics and may have enhanced the microbial degradation. *Bacillus firmus*and *Proteus mirabilis* had the highest degrading abilities.

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