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Investigating the Biodegradation of Low-Density Polyethylene by Proteus and Serratia spp.

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ABSTRACT: Plastics are gradually replacing ceramics, glasses and other materials in their day to day use, their short term application and release to the environment has made them a waste burden on cities and municipalities, where they cause environmental pollution and esthetic damage. There is therefore, a need to develop an eco-friendly protocol to manage plastic wastes. The role of microbes in bioremediation has been established, although, there is paucity of information on the use of Proteus and Serratia spp. to degrade plastics. Hence, the ability of Proteus and Serratia spp. to degrade low-density polyethylene was investigated. The microorganisms were isolated from waste dumpsites in Owerri Municipality and its immediate environs. 100ml enrichment cultures were dispensed into 10 labeled conical flasks, 1ml aliquot of 10^{-1} microbial dilutions of each organism were seeded each into 5 sets of the conical flasks and 0.5g of polyethylene powder was added as the sole source of carbon to each set of flasks, the set-up was incubated in a rotary shaker at 30^0c and 150rpm for 60 days. The optical density (OD_{600}), temperature and P^H changes were measured at 10 days interval, while enumeration of viable colonies in (cfu/ml) during incubation period was also carried out. Results obtained showed progressive but gradual increase in optical density, temperature and P^H values and appearance of air bubbles in the test media indicating microbial activities and ability of the organisms to utilize the substrate as energy source. The viability count of the isolates during and at the end of the incubation period also showed gradual increase in viable colonies (cfu/ml). It is concluded that both Proteus and Serratia spp. were able to utilize the low-density polyethylene as sole carbon source, hence suggesting their ability to degrade the polymer.

KEYWORDS: polyethylene, biodegradation, optical density, enrichment culture and microbes

INTRODUCTION

Plastics are polymers synthesized from fossil resources, and possess advantageous characteristic such as light weight, durability, hardness, and easy processibility. (Longo *et al.*, 2011, Yoon *et al.*, 2012). They are hydrophobic and biochemically inert with high molecular weight, making them recalcitrant to biodegradation, hence they accumulate in the environment and pose great ecological threats (Bhardwaj *et al.*, 2012). Plastics are gradually replacing ceramics, glasses, wood, metals and paper in most of their uses (Mohan and Suresh, 2015).

The most widely used plastic is polyethylene, (Bhardwaji *et al.*, 2012, Azeko *et al.*, 2015). Global polyethylene production had been on the increase, with an estimated 140 million tons in the early part of the 20th century to 409.3 million tons in 2022 (Shah *et al.*, 2008 and Nanda *et al.*, 2010), leading to increased waste burden on cities and municipalities, due to its short-term applications, after which they are discarded and released into the environment.

In most cities of developing countries, there are no effective solid waste management systems resulting in the accumulation of the polyethylene waste along major roads, streets, open space landfills, in drainages and water bodies. Being recalcitrant to biodegradation, they linger for several years if left untouched, with an estimated 25 million tons accumulating in terrestrial and aquatic environments, (Krieg and Holt, 1984 and Mohan and Muresh, 2015) and have been incriminated in environmental pollution, degradation and esthetic damage.

The presence of micro plastics and toxic pollutants has been detected in aquatic food chain, and has resulted in the death of many aquatic animals including fishes (Helden, 2010; Yoon *et al.*, 2012). Similarly, plastics materials buried in the soil reduce water penetration and make the soil unsuitable for agricultural purposes. (Yang *et al;* 2011; Alshehriel, 2017 and Halden 2010). Management of plastic waste has become a major global challenge, the most common method employed in developing countries is open dumping and burning, which has its environmental and health impacts.

However, the use of microbes for bioremediation has been recorded (Okafor *et al.*, 2021), but there is paucity of information in its application in the management of plastic wastes.

There is a need, therefore, to develop an eco-friendly plastic waste management protocol using microorganisms to degrade polyethylene and other plastic products, in a process that will breakdown the complex matrix of polyethylene into smaller units which can penetrate into the cell membrane of microbes and be utilized as energy source to produce biomass, carbon dioxide and water (mineralization).

MATERIALS AND METHODS

Polyethylene Sample Preparation

Low-density polyethylene powder water was sourced from Green Pasture Polyethylene Company, Port Harcourt, Rivers State while sachets for packaging water used in biodegradation assays were obtained from Holy Family Table Water factory, Akwakuma, Owerri North LGA, Imo State. The sachets were shredded and then ground with a grinding machine

Preparation of working Stock

Proteus spp. and Serratia spp. employed in the biodegradation assay were isolated from waste dumpsites in Owerri Imo State Nigeria, using the method described by Gilan *et al.*, (2004) and modified by Azeko *et al.*, (2015). Loop full aliquot of each organism was used as described by Cheesbrough, (2006) to make 10 fold serial dilutions and stored in a well labeled specimen bottle.

Polyethylene Degradation Assay

100ml aliquot of Mineral Salt Vitamin (MSV) medium was dispensed in I0 conical flasks as described by Gilan *et al.*, (2004) and modified by Azeko *et al.*, (2015), after which 1ml aliquot of each of the10^{-1} microbial dilutions was inoculated into each set of 5 flasks and 0.5g of the polyethylene powder was also poured into each sets of the flasks and cultured in a rotary shaker at 30^0c and 150rpm for 60 days.

Microbial growth, P^H and temperature were assessed every 10 days respectively using optical density (OD_{600}) measured with spectrophotometer, P^H meter and thermometer. Isolation and enumeration of potential viable colonies (cfu/ml) was achieved by using MacConkey and Nutrient agar as described by Cheesbrough, (2006).

RESULTS

Table 1: Mean and Standard Deviation of changes in temperature (0^C)

Organisms	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
Proteus spp.	26.4±0.6	26.9±0.3	30.1±0.2	31.0±0.6	32.3±0.8	33.0±0.6	33.5±0.3
Serratia spp.	28.2±0.4	28.7±0.2	30.4±0.1	31.3±0.2	32.2±0.4	33.3±0.7	34.3±0.5

Table 2: Mean and Standard Deviation of changes in P^H

Organisms	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
Proteus spp.	7.11±0.1	7.58±0.6	7.71±0.4	8.60±0.6	9.91±0.5	9.99±0.1	10.39±0.4
Serratia spp.	7.03±0.4	7.81±0.2	8.10±0.7	8.37±0.3	8.93±0.2	9.08±0.3	9.20±0.6

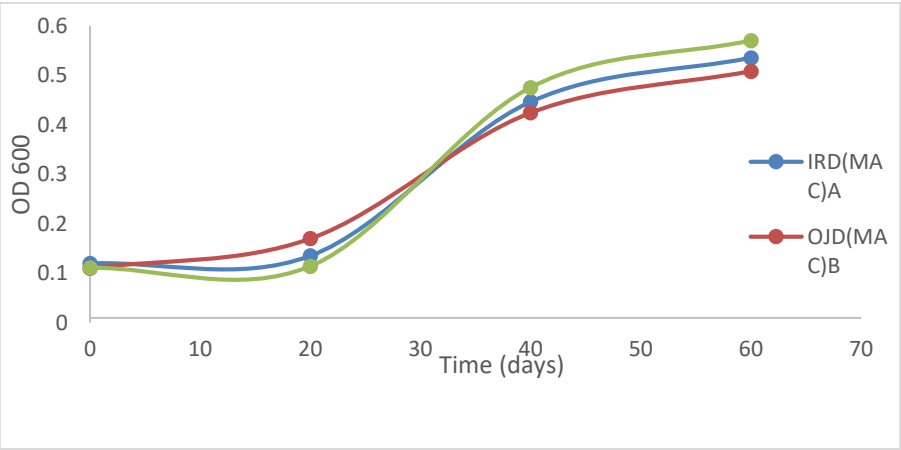

Figure 1: Mean of Growth Measurement (OD_{600}) of Serratia spp. on media containing polyethylene powder.

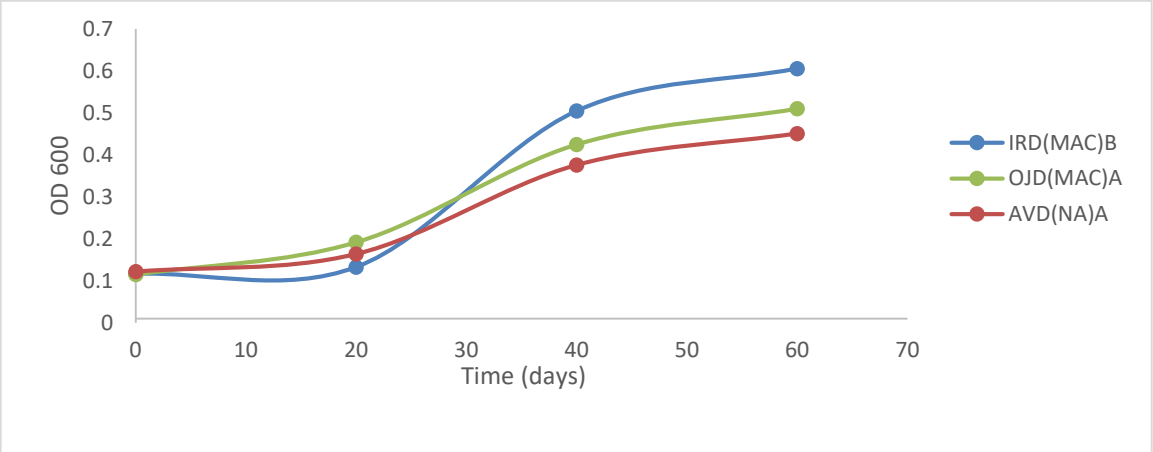

Figure 2: Mean of Growth Measurement (OD_{600}) of Proteus spp. on media containing polyethylene powder.

Table 3: Mean and Standard Deviation of Viable colony count (cfu/ml)

Organisms	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
Proteus spp.	2.04×10^3 ±3.00	1.97×10^3 ±1.50	0.17×10^3 ±1.23	1.53×10^2 ±0.53	2.97×10^2 ±1.11	3.07×10^2 ±1.43	4.83×10^2 ±1.53
Serratia spp.	2.64×10^3 ±4.04	1.03×10^3 ±2.53	0.07×10^3 ±1.33	4.77×10^2 ±2.25	4.23×10^2 ±1.63	5.57×10^2 ±1.03	6.97×10^2 ±1.53

DISCUSSION

The ability of Proteus spp. and Serratia spp. to degrade low density polyethylene was determined by measuring the growth of the organisms using optical density (OD600), temperature and pH readings taken at interval of 10 days during incubation. Our result indicated a 0.5 increase in mean temperature for Proteus spp. and 0.7 for Serratia spp. at day 10 of culture (See Table 1), this negligible increase could be as a result of the microbes adjusting and acclimatizing to the new environment and initiating capabilities to utilize the polyethylene as sole carbon and energy source. However, between day 30 and day 60 of culture, there were almost a steady 1^{0C} rise in temperature for the two microbes (See Table 1); this characterizes very slow microbial activity and collaborates Yang *et al.*, (2011) who reported very slow microbial degradation of plastic material.

The mean P^H at day zero for the two organisms were neutral, 7.11±0.1for Proteus and 7.03±0.4 for Serratia spp. (See Table 2), however, as days of culture increases, the P^H continued to increase as the growth medium became alkaline and peaking at 10.39±0.4 for Proteus and 9.20±0.6 for Serratia spp at day 60 (See Table 2). This data indicated microbial degradation of polyethylene, through its usage as carbon source and secretion of waste product that increased the P^H value of the culture media. This is consistent with the observations of Das and Kumar (2015). Optical density is used to estimate the concentration, growth and metabolic activities of microbial cells in a liquid or concentration of a solution of organic molecules, and OD $_{(600)}$ is used because cell damage is minimized at this wavelength.

The result of the optical density for both Proteus and Serratia spp., showed decrease in value at day 10 (See Figure 1 and 2), this could be as a result of the microbes struggle to adapt and switch to the usage of the polyethylene as source of carbon and also death of the cells that were not capable of utilizing the polyethylene as energy source. Lalit (2013) reported similar results using five different bacterial strains isolated from compost suspension to degrade polyethylene and polypropylene. As the period of incubation progressed, the media became turbid with increased in the OD reading (See Figure 1 and 2), and gas bubbles were observed, indicating microbial metabolic activity and usage of the substrate, similar observations were recorded by Azeko *et al.*, (2015) who used Serratia marcescens and its cell free extract in linear low-density polyethylene biodegradation.

The findings of this study also corroborates earlier findings by Esmaeili *et al.*, (2013), Azeko *et al.*, (2015), Longo *et al.*, (2011), Patil and Bagde (2015) and Ren *et al.*, (2019) who used *Lysinibacillus xylanilyticus* and *Aspergillus niger*, *Serratia marcescens* and its cell free extract to degrade plastic materials. Optical density is used to estimate the concentration, growth and metabolic activities of microbial cells in a liquid or concentration of a solution of organic molecules.

Furthermore, the survival of the microbes in the media with polyethylene as the sole source of carbon and energy after 60 days of incubation is an evidence of their ability to degrade and utilize the polymer as its carbon source (See Table 3), although, there was a significance decrease in the bacterial populations between day 10 and 20 (See Table 3), this could be attributed to death of the cells that were unable to utilize the polyethylene as energy source, however, as incubation period increased, there was a very slow decrease of viability,

peaking at 4.83×10^2 ±1.53(cfu) for Proteus and 6.97×10^2 ±1.53(cfu/ml) for Serratia spp. at day 60 (See Table 3).

CONCLUSION

This study has observed that Proteus and Serratia spp. are able to adapt and utilize low-density polyethylene as carbon source, this suggests that other low-density plastic materials could be degraded these organisms. This data may disabuse the generally accepted myth that plastics are non-degradable. Although, environmental factors play important role in plastic biodegradation as their intercellular matrix, their mechanical strength and hardness are weathered. Generally, plastic biodegradation is slow, different pretreatment measures may be required to improve the process. It is concluded from this study, that some indigenous microorganisms could be deployed for the degradation plastic materials under favorable conditions.

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