A COMPARATIVE STUDY ON BIOLOGICAL DEGRADATION OF PLASTIC FROM ISOLATED MICROBES USING VARIOUS METHOD

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ABSTRACT

Plastic eradication is one of the most important need in our life and it becomes necessary factor to lead our healthy generation. Plastic is widely used as various products like plastic bags, plastic bottles and pipes among people. Meanwhile, enormous usage of plastic leads to environmental threats. The existing method of Plastic degradation achieved by physical chemical and biological method. In recent year plastic degradation by microbes were the important research area where the application of various microbes and various methods were analysing. Bacteria and fungi play vital role in the degradation process. The present study aims polyethylene, polypropylene and polyvinyl chloride biodegradation using four different method such as artificial media method, modified ASTM G21 70 method, dip hanging method and Compost burial method. The five bacterial and three fungal strains were isolated from compost which is prepared from mixture of cow dung, vegetables waste, Paddy's straw, and saw dust. After that isolates were allowed to characterization test such as staining, biochemical test and antibiotic sensitivity test, isolates allowed for degradation property. Out of eight isolates, white and green fungi highly degrade the polyethylene with 78.8% of weight loss, polypropylene highly degraded by creamy bacteria with 20.4% weight loss, then, polyvinyl chloride highly degraded by black fungi with 4.6% of weight loss. Besides weight loss method, morphological changes was identified by FTIR and SEM analysis.

Keywords: Biodegradation, compost, modified ASTM G21 70 method, dip hanging method, polyethylene, polypropylene, polyvinyl chloride.

INTRODUCTION

Plastics are high-molecular-weight organic polymers. Plastics are typically produced by human industrial processes. These polymers are produced from a variety of hydrocarbons and petroleum derivatives (Abraham et al., 2016) and various renewable materials, like cotton or corn derivatives, are utilized in current industrial processes. According to Kale et al., in 2015 plastics are polymers that can be moulded into moulds by heating them up except for biodegradable bioplastics (Akmal et al., 2015). Plastics versatility makes them an

essential material in different applications; they are used in packaging (39.9%), construction (19.8%), the automotive industry (9.8%), electronics and communication (6.2%), agriculture (3.4%), household leisure and sport (4.1%), and other fields (16.7%). Among the different plastics, the most commonly used in packaging are polypropylene 4 (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), vinyl chloride (PVC), polyurethane (PU), polyethylene terephthalate (PETE), and polystyrene (PS).

According to Plastic Pollution (2014), PE is linked to hormonal disturbances, developmental issues, cancer, decreased sperm count, infertility, and diminished immunity. PVC, a type of plastic used in cosmetics, containers, packaging, and utility items, has been linked to cancer, birth defects, and other genetic conditions. Additionally, it may cause problems with 6 digestion and the liver, bronchitis, skin disease, deafness, and vision. Mercury, dioxins, and phthalates, among other dangerous poisonous chemicals released during the PVC life cycle, may pose health risks that cannot be reversed over time. Dioxins, one of the most potent synthetic chemicals ever tested, are released when PVC plastic is made or burned. These dioxins can cause cancer and harm the immune and reproductive systems. According to Zeenat et al (2021), the compound bisphenol A, which is found in some plastic water bottles, is thought to cause cancer, weaken the immune system, cause early puberty, and lead to the development of obesity and diabetes.

In past years polyethylene degrading bacteria has been reported such as, Acinetobacter baumannii, Arthrobacter spp, Viscosus spp, Pseudomonas spp, Arthrobacter viscosus, Bacillus amyloliquefaciens, thuringiensis, mycoides, cereus, pumilus, Staphylococcuscohnii, Xylosus spp, Pseudomonas fluorescens, Rahnella aquatills, Micrococcus luteus, lylae, Paenibacillus macerans, Flavobacterium spp, Delftia acidovorans, Ralstonia spp Rhodococcus erythropolis, Pseudomonas aeruginosa [Koutny et al., 2009] and Bacillus brevies [Watanabe et al., 2009]. The purpose of this study was to isolate microorganisms from compost and screen for the potential plastic degrading microorganisms and identifying the high potential microorganisms that degrade plastic.

MATERIALS AND METHODS

The cow dung, saw dust, paddy’s straw, egg shells and vegetable waste are collected from mahar nonpu pottal, kenkarai, Ramanathapuram district.The collected waste materials were allowed to be dried and chopped into small materials. Next, a plastic container was prepared with a hole for aeration. The collected waste materials were added one by one and mixed with the materials. The prepared plastic container was placed in the shaded area and leaves to composing process bacteria isolated and characterized by biochemical test.

Muller hinton agar was prepared. A petri dish and five bacterial and three fungal broth cultures were obtained. Swab was taken and dipped into broth cultures. Using a dipped swab, streak on the plates in even to obtain a uniform growth. Plates were rotated and reswabed. Using sterile forceps, the antibiotic disc (amoxycillin, ampicillin, pencilin G, gentamycin, vancomycin, streptomycin, kanamycin, nystatin) were taken aseptically and then placed on plates. Plates were incubated at 37℃ for 24 hours. Results were observed that zone of inhibition was noted (Albertsson,, 1980).

Three types of plastic samples were selected, such as polyethylene, polypropylene, polyvinyl chloride. These three samples were more harmful to the environment as well as human health.Biodegradation carriedout by artifical method (Montazer et al., 2020), Dip Hanging method (Alshehrei, 2017) and ASTM G21-70 method (Alshehrei, 2017).Plastic samples were obtained after 15 days of incubation. Samples were washed using 2% sodium lauryl sulphate to remove bacterial and fungal biomass, dried overnight and then 28 weighed. The weight loss of the sample was measured by weighing samples before inoculation as initial sample Weight (Wo) and after 15 days of inoculation process (Wf). Per cent weight loss was calculated.

RESULT AND DISCUSSION

A plastic container was filled full of waste material on the 1 st day. But on the 28th day the mixed materials were converted into a small powder format, a large space appeared on top of the container and an earthy smell developed. Biochemical tes results of isolated microbes were noted in table 1.

Antibiotic sensitivity was used to decide how powerful an antibiotic was against the test life form. The eight separate one were screened for susceptibility towards eight anti- toxins (pencillin, gentamycin, vancomycin, nystatin, streptomycin, amoxycilin, kanamycin) and the outcome was noted. Half white (smooth), white, slight yellow, black and white and green fungi have no antimicrobial movement (Table 2).

All plastic samples were pre-treated with 70% ethanol.

In the artificial media method, polyethylene, polypropylene and polyvinyl chloride pieces were added at an initial weight of 0.0142 g, 0.2328 g and 1.5700 g along with 1ml of inoculum. There are five selected bacteria and three selected fungi separately into dubos mineral salt medium for bacteria and czapek dox medium for fungi. After 15 days of incubation, each plastic sample was reduced in it’s weight and some color changes were observed. Compared to another study, the ability of selected isolates to degrade LDPE was very similar to that of previous research. After 60 days of incubation in MSMB under shaking

conditions (at 130 rpm) (Munir E et al., 2018), Das and Kumar discovered that Apergillus reduced the weight of LDPE film by 5-8% and Fusarium by 9%. The total weight loss of LDPE has also been found to be significantly higher. According to Singh and COMPOST BURIAL METHOD FIGURE 13: SODIUM LAURYL SULFATE TREATED PLASTIC SAMPLES 74 Gupta, Mucor sp., Aspergillus japonicus, Aspergillus flavus, and Aspergillus niger all reduced weight by 30%, 20%, and 36%, respectively. In this present study, colour changes observed on the polyethylene and polyvinyl chloride samples. The colour of polyethylene sample into the faded and blue colour of the polyvinyl chloride sample changed into grey and white and polyethylene samples degraded into 65.2%, polypropylene samples into 6% and polyvinyl chloride into 25%.

Dip hanging method, the specific media are not used. So, the carbon source for bacterial and fungal growth only comes from plastic samples. After incubation of 15 days of incubation, bacterial and fungal colonies stick on the plastic samples. Compared to another study,the decomposition time for PCL from England and PLA from Japan is 60 days, according to the ASTM standard. In linear polymers, chemical degradation reactions result in a decrease in molecular weight or a shorter chain length. Acetal bonds in starch are very simple to degrade. Because the bioplastic's composition was a natural material that microbes could easily digest, this mass reduction was significant. A natural polymer with a hydroxyl group (-OH) was the primary characteristic of a polymer that could be degraded naturally; consequently, this group was easily degraded by microorganisms (Zeenat et al., 2021), A. niger lost 11.5% of its weight. The dip-hanging method resulted in poor growth (R.C Nissa et al 2019). But in this study, On the 15 th day, polyethylene samples faded and polyvinyl chloride samples were changed to grey and black with some dots appearing and weight also reduced. The weight of polyethylene, polypropylene and polyvinyl chloride is 61.6%, 8.1% and 15 % reduced.

By the ASTM method, organisms take nutrients from glucose salt agar. The inoculum was spread on the surface of the agar plates and plastic samples were placed on it. So, organisms grow around and on the plastic samples. Compared with Alshehrei,2017, in the modified ASTM G21-70 method, A. niger flourished on the bioplastic sample’s surface, indicating that the bioplastic could be degraded by the fungus. In this study after incubation, the weight of the polyethylene samples degraded into 61.3%, polypropylene samples in 19.6% and polyvinyl chloride degraded into 15.8%. But colour change was not observed.

CONCLUSION

The five bacterial and three fungal strains were tested for their ability to degrade polyethylene, polypropylene and polyvinyl chloride. Biodegradation occur four different methods. From this study, out of eight isolates, three strains were well performed after 15 days of incubation. The well performed isolates consist of two fungal and one bacterial strain. Those three are white and green fungi, slight yellow bacteria and black fungi. White and green fungi promising strain to degrade polyethylene, slight yellow to degrade polypropylene and black fungi to polyvinyl chloride degradation. The three well performed strains highly degrade the respective plastic samples at the presence of nutrients such as artificial media method. So, artificial media method was suitable method for plastic degradation.

TABLE 1: BIOCHEMICAL CHARACTERS OF ISOLATES

S.NO	TEST	Bacterial Colonies				
		Pink	Half white, smooth	Half white, rough	White	Yellow
1	Indole	+	+	-	-	+
2	Methyl red	+	+	-	+	+
3	Voges proskauer	+	+	+	+	+
4	Catalase	+	+	+	+	+
5	Citrate	+	+	-	+	+
6	Urease	-	-	+	+	-
7	Starch hydrolysis/ amylase enzyme	-	-	+	+	-
8	Casein hydrolysis/ protease enzyme	+	-	+	+	-
9	Gelatin hydrolysis	+	-	+	+	+
10	Nitrate reduction	+	+	-	+	+
11	Oxidase	+	-	+	+	+
12	Cellulose enzyme	+	-	-	+	+
13	Glucose fermentation	-	+	+	+	+
14	Gas production	+	+	+	+	+

TABLE 2:ANTIBIOTIC ASSAY OF ISOLATED STRAINS

S.NO	ISOLATES	ANTIBIOTIC DISC assay (mm)			
		AMOXYCILIN (30mg)	PENCILIN G (10mg)	KANAMYCIN (30mg)	STREPTOMYCIN (10mg)
1	Half white, rough	14	-	-	18
2	Pink	14	-	-	-
3	Half white, smooth	-	-	-	-
4	Yellow	-	-	-	-
5	White	-	-	-	-

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