Synthesis and Bio Degradation of Bio-Plastic Prepared from Corn and Potato Starch

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Abstract
Plastic today is derived from petrochemicals which are produced from fossil gas and oil. The widespread presence of plastic and plastic particles in the environment has deleterious and profound effects on the living organisms. The enormous amount of plastic has been accumulated in open oceans, terrestrial environment, on shore lines and even deep sea. It has been persisting in the environment for hundreds to thousands of years and posing a serious threat to wild life and marine organisms by chocking and releasing toxic chemicals. Plastic is hard to degrade due to its structure and its derivation from sources like fossil fuels. In the present study, bio-plastic is being prepared using starch which was extracted from raw potatoes and commercially available corn starch. The starch is mixed with acetic acid and propane 1,2,3-triol to prepare bio- plastic. The use of the bio-plastic is an effective alternative as it is biodegradable by the environmental microorganisms. Microorganisms such as species of bacillus are found to degrade bio-plastic naturally. Bacillus is a gram positive, rod shaped common bacteria present in the soil. Findings conclude that bio-plastic can easily be prepared and environmentally present organisms can degrade it.
Keywords: Bio-plastic, Bacillus, Starch, acetic acid, Propane 123-triol, microorganisms
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INTRODUCTION

Pollutants enter our environment naturally or through human activities. Natural pollutants disperse in the environment breaking into non harmful particles by natural processes. Most serious pollution forms are due to human activities that occur in or near urbanized areas or industrial areas. The point source pollutants come from single identifiable sources, such as the drainpipe of a meat- packing plant or the chimney of a house. While non point source pollutants enter the soil, water, air from hard to locate sources. Two kinds of wastes are identified in the environment degradable wastes are easily degraded naturally by the act of nature and microorganisms and the non-degradable wastes persist in the environment for millions of years. The best way to deal with non-degradable pollutant is to reduce their use and reuse them and limit their dumping into the environment [1].

Municipal solid wastes are the largest type of waste generated throughout the world which consists of inorganic and organic pollutants. The organic solid waste generated from municipal sources can further be dividing into 3 categories: liable, fermentable, and non-fermentable. Liable wastes include materials such as foodstuff that decompose fast. Fermentable wastes decompose rapidly, while non-fermentable waste tends to resist decomposition and, hence break down very slowly [6].

Inorganic solid waste includes products like plastics, metals and other non-biodegradable materials. In term of toxicity, few solid wastes are classified as hazardous which include pesticides, electric waste, herbicides, medical waste and paints are recommended to be discarded of in special ways and not to be mixed with general municipal waste. In developing countries solid waste characteristically has a high content of organic waste compared to that in developed countries, because in developed countries there are ways by which organic waste is utilized and less organic waste is generated. In developed countries there is more electronic waste and inorganic waste [6].

In the natural world, wherever dominance of humans is less, there is essentially no waste because one organism waste becomes nutrients for others [2]. About all consumption and processing activities of people, from industry to commerce, agriculture and health generate solid waste. It is estimated that a person generates about 1600 kg of waste every year in a developed country. Solid waste is often considered third pollution after water and air. It is that material that arises from varies activities of human and which is normally discarded as unwanted or useless. It highly consists of heterogeneous mass of materials discarded from urban community and more of homogenous waste accumulated from agricultural, industrial and mining waste. Solid waste is typically classified into further groups on bases of heating and moisture value. These classifications are: rubbish, garbage, pathological waste, industrial waste and Agricultural waste [4]. Solid waste can be dealt with in various ways. A way is by reduction of waste by which much less waste and pollution is produced, and the waste produced are considered to be potential resources that can be recycled, reused or composted, many scientists think that 75- 90% of solid waste can be eliminated by using these strategies. Another perspective is waste management in which we manage waste in ways to minimize their environmental harm without trying seriously to reduce the amount of produced waste. It involves mixing of waste and then transferring them from one environment to another, usually by burning or burying them or shipping them to another location [1]. Solid waste generation and urbanization are much related and hence it is important to shortly reflect on the phenomenon of urbanization. In 1950's, almost 30% of the world population was living in urban areas. It is presently estimated that till 2050 about 66% of the world's population will be urbanized [6].

The impact of solid waste on the health of living organisms differs and may be dependent on vast factors which include: the population exposed, the duration of exposure, nature of the waste and availability of prevention and mitigation interventions. The impact may range from mild psychological effects to severe mobility, disability or even death. Exposure impacts of solid waste on living organism health are weak and inconclusive in various cases due to hurdles encountered in perfectly ascertaining exposure, controlling of confounders, accounting for the exposure duration and inability to follow up those exposed to ascertain outcomes that do not manifest in short term [6].

One of the most visible and long lasting changes to the Earth's surface is the fragmentation and accumulation of plastic [7]. Plastic has been a great invention of the 19th century, brought great ease to the people [3]. Humans have benefited by the use of polymers since around 1600 BC when first natural rubber was processed into balls, figurines and bands by ancient Mesoamericans. In the past years, humans have relied increasingly on rubber and plastic, first experiments were with polymers, horn, waxes, natural rubber and resins, until the nineteenth century, when modern thermoplastic began to develop. In 1839, vulcanized rubber was invented by Goodyear, and a German apothecary, Eduard Simon, discovered polystyrene. Through the 19th century the developmental work continued on natural and synthetic polymers producing notables such as celluloid for billiard balls, polyvinyl Chloride, which is used in myriad execution and viscose (rayon) for clothing. The first 50 years of 20th century was really expanded by the development of modern plastic, with at least 15 new polymer classes being synthesized [8]. During the last few years the plastic industries have developed new polymers, because of which the risk of hazard have introduced [9]. The plastic success as a material has been substantial; they have proved to be versatile for wide use range of types and forms, including natural polymers, modified natural polymers, thermosetting plastics, thermoplastic and most recent addition the biodegradable plastic. Plastics have numerous unique properties; they are chemical and light resistant, can be used at a very wide temperature range and they are strong and tough, but as a hot melt can be easily worked. It is the low cost of plastic and the range of properties that has driven the worldwide annual demand for plastic to reach 245 million tons today. At a 5 per cent conservative growth, a continuation of this trend suggests that amount of plastic at least about 308 million tons will be consumed annually worldwide by 2010. This projected growth is mainly because of the increasing public demand for plastics [8]. Most of the human societies have been facilitated by the use of plastic over the past century. Plastics are made up of a network of molecular monomers that are bounded together to form macromolecules of infinite use in the human society. At present, there are more than twenty different major types of plastic in use worldwide [5].

Plastic are lightweight, inexpensive and durable material, which can be shaped readily into a variety of products that are found to be used in a wide range of applications [10]. Plastics are remarkably flexible materials that provide considerable benefits as simple as packaging to complex engineering material, traditional synthetic polymers (called often as plastics), such as polypropylene and polyethylene have been synthesized from non-renewable petrochemicals and are known to cause environmental concerns due to their nature of non-biodegradability [11]. Around 4% of oil and gas production of the world, a non-renewable resource, is used as feedstock for the plastic and about 3-4% more is expended to provide energy for the manufacturing of plastic. A large portion of plastic produced each year is utilized in making disposable items of packaging and other products that are discarded within 1 year of manufacture. These 2 observations indicate that our current use of plastic is not suitable. Due to this much ease of plastic materials the production of plastic has increase remarkably over the past 60 years [10]. About hundred and forty million tons of plastic are utilized every year worldwide, which requires the processing of about 150 million tons of fossil fuels and causes huge amount of waste that can take about thousands of years to naturally deteriorate, if it degrades at all. The alternative of plastic is bio degradable plastic, it has the same strength but can degrade easily without posing a threat to the environment [12].

In the previous half- century, there have been many changes on the surface of Earth, but the most instantly observable one is the abundance of plastic debris. Like many human caused impacts on natural systems, it is one that, in spite of widespread recognition of the issue, is still expanding and even if stopped immediately will persist for centuries to come. From what began as a perceived aesthetic problem of plastic littering countryside, towns, shores and even the vast spread ocean soon emerged as causing the chocking and tangling of wildlife. The figure of potentially harmful implications of plastic debris that have been observed has enhanced and it is now realized that these materials may also carry and transport persistent organic pollutants (POPs), non- indigenous species to new locations and scatter algae associated with red tides [7].

On Earth where there are finite resources, the need to employ practices which motivates reuse, recycling and a return of resource to natural cycle, becomes an essential and sustainable target. The utilization of synthetic

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polymers and their disposal has manufactured a scourge of material incapable of deteriorating or composting within logical time periods. Thus, the utilization of degradable or compostable polymers forms the foundation of best practice with the environment's health in mind. Polymers skilled of degrading can be broken down into following: degradable, biodegradable and compostable. PHA's are one of the most recognised biodegradable plastic ever manufactured which produces zero toxic waste and is capable of recycling completely into organic matter which is recyclable [13].

The search for biological plastics dates back from 1913 when a British and a French scientist filed independently for patents on plastic synthesised from soy. At the time intense competition was there between petrochemical and agriculture industries to dominate the market for plastics derived from organic polymers. Oil was widely available so petrochemical plastic took over the market. Now with the projected climate change and other environmental problems linked with the use of oil and other fossil fuels, the chemists are stepping up efforts to make environmentally sustainable and biodegradable plastics from a variety of green polymers. Such bio-plastics can be synthesised by corn, soybeans, switch grass, chicken feathers, sugarcane and some components of garbage. The CO_2 extracted from the coal burning power plants could be utilized in this process. Manufacturing such biopolymers requires finding suitable chemicals called catalysts, which accelerate the reactions that chemists can use to produce polymers from biologically based chemicals without applying high temperatures. With proper design and mass production, bio-plastics could be stronger, lighter and cheaper, and the process of manufacturing them could require less energy use and result in producing less pollution per unit of weight which is less than manufacturing of conventional petroleum based plastics. Instead of being sent to landfills, packaging and materials made from bio-plastics could be composed to produce a soil conditioner, in recycling the nutrients and keeping it returning back to the environment [2].

Biodegradable plastics can be split into 3 groups according to their production: (1) polymers that are chemically synthesised, (2) Polymers that are derived from plants and (3) bacterial polymers that can be synthesised by microbial fermentation or bacterial bio-film. The polymers derived from renewable sources such as microorganisms and plants, are maintainable ecologically because they are not accumulated in the environment for a long time period and they are deteriorated or mineralized by microorganisms naturally present. However, these polymers have some physiochemical properties that restrict their use. Chemically inhanced biodegradable plastics may be divided into 2 groups: (1) those which are synthesised by the degradation of structures of chemical by the direct action of enzymes, such as cellulose and amylase, and (2) those that are manufactured degradable by the action of only one or various physic-chemical processes, for example: photolysis, hydrolysis or pyrolysis [14].

Biodegradable plastic belong to the family of polymer products with a molecular structure that is liable to biological degradation into harmless or even beneficial products. The environmentally favourable opinion of these materials and their range of application are expanding: it already extends to mulch films, silage wrap, landfill covers, composting bags, seed coating, bundling strings, planter boxes, fish nets, packaging of agriculture supplies and pellet coating for delayed release of herbicides, pesticides and fertilizers. The rate of decomposition of bio-plastic in the environment is controlled by chemical structure of the plastic, as well as by the environmental conditions such as humidity, temperature and nutrient content, all of which persuade microbial activity. The biodegradation rate of particular bio-plastic is still difficult to find in a given situation and the materials often persist longer than they are needed. Great reliability is required due to increasing use of biodegradable plastic, and one way by which degradation may be achieved is to understand the efficiency of enzymes in degrading biodegradable plastic and the natural habitat of the microorganisms that produce these kinds of enzymes [15].

Polyhydroxyalkanoates (PHAs) a unique class of optically active biopolymers is the most popular bio-plastics and is a potential contender in replacing some plastics synthesized by petroleum because of their biodegradable nature and physical properties. PHAs are somewhat similar to the well-known synthetic polymers that are low density polypropylene and polyethylene; furthermore the disposal of PHAs as bio-waste made them more attractive in the hunt of sustainable development of bio-plastic. These PHAs contained a class of polyesters naturally present which has been accumulated by many microorganisms intracellularly in form of granules and stored in response to nutrient limitation or environmental stress as a carbon reserve, energy and reducing power [11]. Polyhydroxyalkanoates (PHAs) are deposited as energy and carbon materials by many bacteria under unbalanced growth conditions [16].

Bio-plastic can be prepared from poly (β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV) or cornstarch or a blend of both PHBV and cornstarch. PHAs are microbial polyesters which have been receiving a lot of attention as biodegradable alternative to petroleum based plastics. Copolymers of hydroxyvalerate and hydroxybutyrate including PHBV form plastics which have good quality and have been commercially marketed. Many articles have been published pertaining to the manufacturing, application and properties including biodegradability of PHBVs. The wide use of PHBVs is still questioned due to its high cost than the conventional plastic, despite of its environmental benefits [17]. Bio-plastic can also be synthesized by microbial production heterotrophy, which is an effective method due to its greater product flexibility and productivity compared to the ones obtained by photoautotrophy. Nevertheless, such heterotrophic systems, which are mainly established in bacteria, relay on a significant consumption of composite organic compounds. Therefore a heterotrophic approach that requires a less amount of a plain organic substrate is more advisable [18].

In some recent years, study in the field of biodegradation has paid attention on organic recycling like the biodegradability in composting plants, as bio-plastic products are considered to be recovered at the end of their life through organic recycling with the production of compost [19]. Most synthetic polymers that are synthesized from petroleum, biodegradable polymers, when disposed in the environment, may primarily be cut from the polymer chain by non-enzymatic processes such as chemical hydrolysis, photolysis and subsequently degrade by enzymes released by bacteria, fungi and algae. The biodegradable polymers after enzymatic degradation can be converted into methane, carbon dioxide, biomass, humus, water and other substances [14].

The biodegradable plastic's degradability depends on the organisms present in the environment that can degrade it. Though, very less is known about which organisms are responsible for the degradation of bio-plastics in-situ. Species of bacillus are found to biodegrade plastic according to studies, soil biodegradation of plastic is observed by Brevibacillus reuszeri spp. [20]. Pseudozyma spp. Strain is studied to secrete a biodegradable- plastic degrading enzyme [15]. The aim of presented study is preparation of bio-plastic using corn and potato starch with strength and flexibility and its natural degradation by species of Bacillus which are environmentally present.

MATERIALS AND METHOD:

MATERIALS:

- Glassware
- Aluminium foil
- Grater
- Mortar and pestle

REAGENTS:

- Propane 1,2,3-triol
- Acetic Acid
- Safranin
- Iodine
- Ethyl Alcohol
- Crystal Violet
- MacConkey Agar
- Nutrient Agar
- Blood Agar
- Nutrient broth

METHODS

SAMPLE COLLECTION:

- 100 grams corn starch was purchased from the local super market.
- Raw potatoes were purchased from local vegetables stall.

EXTRACTION OF POTATO STARCH:

- Potatoes are peeled and grated using grater.
- The potatoes are kept in a mortar and 100 ml water is added and crushed with pestle.
- The water is filtered in another beaker and allowed to rest aside for 20 minutes.
- The water is poured off and starch is left at the bottom.
- Additional 25 ml water is added to the starch and stirred with a glass rod and left for 20 minutes to clean the starch.
- The water is poured out and starch below is left to dry.

PRODUCTION OF BIO-PLASTIC:

- 14.3 grams of starch, 0.1 L of water, 29.5 ml of acetic acid and 29.5 ml of propane 1,2,3-triol was added together in a beaker.
- The mixture was stirred till it thickened under Bunsen burner.
- The mixture was spread on an aluminium foil to dry at room temperature.
- Transparent plastic was obtained after 24 hours.

ISOLATION OF BACTERIA FROM DEGRADING BIO-PLASTIC:

- Cotton Swab was dipped in Nutrient broth and culture was taken from the degrading plastic using the cotton swab.
- Culture was inoculated in the Nutrient agar plate and kept in the incubator for 24 hours at 28°C.
- Next day colonial morphology was noted and through gram staining bacteria was identified under oil

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immersion lens of power 100x.

- Sub culturing was done by inoculating colonies into MacConkey agar, Nutrient agar and Blood agar plates for 24 hours at 28°C.
- Next day colonial morphology was noted and through gram staining bacteria was identified under oil immersion lens of power 100x.

RESULT:

Bio-plastic was prepared using commercially available corn starch and potato starch extracted from raw potatoes as shown in Fig 1, 2 and 3. After some time the plastic started to degrade and show zones of clearance as shown in Fig 4.

Fig 1: Prepared bio-plastic

Fig 2: Prepared bio-plastic

Fig 3: Prepared bio-plastic

Fig 4: Degradation of plastic at zones of clearance

Culturing was done from the degrading bio-plastic onto 3 types of Agar namely Nutrient agar, Blood Agar and MacConkey agar. The colonial characteristics and microscopic characteristics are shown in Table 1, Fig 5, 6 & 7 and in Table 2 & Fig 8 respectively.

Table 1: Colonial Morphology of degraded plastic

TYPE OF AGAR	COLONIAL MORPHOLOGY
Nutrient Agar	Circular, flat, undulate margin and cream coloured colonies
MacConkey Agar	No growth
Blood Agar	Beta Haemolysis

In Nutrient agar variety of colonies were observed circular, flat, undulated margin and cream coloured colonies. In MacConkey agar no growth was observed. In Blood Agar the blood was completely haemolysed by microorganisms showing haemolysis.

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Fig 5: Bacillus sp. On nutrient agar

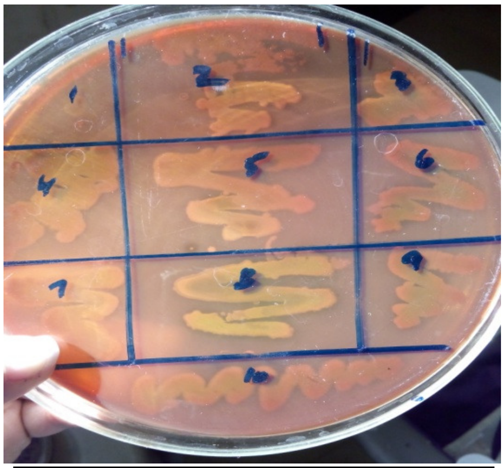

Fig 6: β haemolysis on blood agar

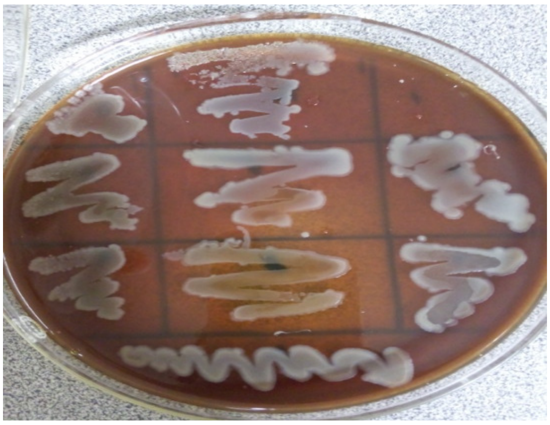

Fig 7: β haemolysis on blood agar

Table 2: Microscopic identification of degraded plastic

CULTURE	SHAPE	ARRANGEMENT	GRAM REACTION
Bacillus	Rod shaped	Scattered and in chains	Gram + (ve)

Under the microscope gram positive organisms were observed. The organism was elongated rod shaped, present in chains and even in separate forms. By the microscopic appearance the bacterial specie found on the degrading bio-plastic was identified as gram positive Bacillus.

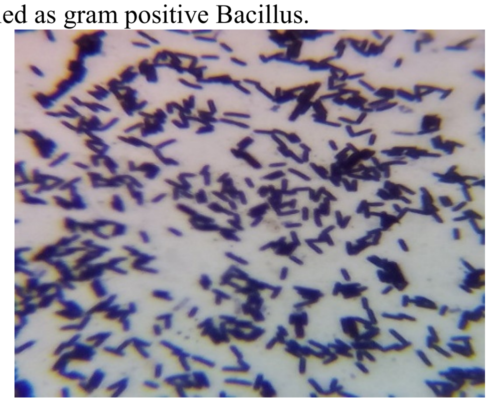

Fig 8: Bacillus under microscope

DISCUSION:

Bio-plastic is a huge step towards a sustainable environment which can be a great way of controlling the solid waste. Degradation of plastic can take place by normally present fungi, bacteria or algae. According to the study bio-plastic was prepared using corn and potato starch. The biodegradable plastic was flexible and strong. A week later degradation was noted visibly on plastic on room temperature 27°C. The visible degradation spots were tested by inoculating them on 3 types of agars namely Nutrient agar, Blood agar and MacConkey agar. In Blood agar -haemolysis was observed, while on Nutrient agar the colonies of gram positive bacteria were observed to be species of bacillus but on MacConkey agar no growth was observed. According to a study conducted by Catherine Anne W. on degradation of bio-plastic the results show that microbial film appeared after 9 days of burial of bio-plastic in controlled environment at soil temperature ranging from 11°C at night and 30°C at day while in the presented study at 27°C room temperature the degradation was observed. This could be due to the difference in the surrounding conditions, temperature variation, raw material use and environmental conditions that in this study the degradation occurred much faster.

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