



A Review on Bioplastic Production - A Need to the Society

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ABSTRACT

The utilization of plastics was increased worldwide and creates a serious pollution issues when disposed of directly in environment. The researcher alarmed with increased utilization of plastics and its deleterious effect to the globe for the future generation. The plastics are replaced by numerous varieties of bioplastics. This research article mainly focuses on utilization of substrates such as Pomegranate peel, Potato, Orange peel and Water hyacinth as a potential substrate for the production of PHA by using various organisms. The bioplastics are made for the replacement of synthetic polymer, which is easily degradable and environment-friendly. Market demand for bioplastic is growing due to consumer- friendly products. It is less associated with conventional plastics production than other bioplastics.

Keywords: Biopolymer, Conventional plastics, Polyhydroxyalkanoates.

INTRODUCTION

Plastics are long chain man-made polymeric molecules that are inexpensive, lightweight, and durable¹. The living organism in the ocean and the surrounding get affected everyday due to the raise in plastic pollution and the effects are called as 'Trash islands' or the 'garbage patches'². The macroplastics are converted into microplastics for which it takes at least 400 years to break into small fragments³. The Chlorinated plastics releases the harmful chemical to the soil which enters into ground water. This is harmful to species that intake those chemical mixed water.

The serious issue warned the scientist to look for the alternative to plastic and resulted in the production of biomasses. Currently, over a decade, variety of bioplastics are available in the market⁴. Currently, biodegradable plastics only represents a small market with conventional petrochemical products⁵. Many microorganism are capable of degrading the bioplastics in different pathways based on the nature of production, additives added to the polymer and the properties like crystallinity, molecular weight etc.,⁶.

1. Effects of Conventional Plastics

The adverse effects of plastics on human populations associated with emerging literary system pose health risks. It is found that the toxic chemicals are employed in the production of conventional plastics. Biomonitoring helps to indicate the human population explosion to the chemicals level used in plastic production⁷. This biomonitoring process helps to find out the adverse effects including reproductive abnormalities in human population⁸.

Plastics are shed in the ocean and they accumulate at sea level and form a bed-like layer on the sea bed, which in

turn affects fishing, shipping and other marine activities^{9,10}. Marine microorganisms are rapidly colonized on the free floating plastic debris and can lost in the sea surface for considerable period of time in the native form¹¹. This warned the public lovers who cares the aquatic organisms and medias took keen interest to entanglement and ingestion by wildlife and to monitor the spatial and temporal patterns of plastic debris on the regional sea surface¹².

2. Bioplastics

One of the green materials emerging in market was known to be Bioplastic¹³. Bioplastics are named as it produced from biomass and hence it is also known as biodegradable plastics. Bioplastics is a long chain of monomers linked to each other by ester bonding. Plastics are considered as polyesters. This Bioplastic is a plastic from renewable sources such as vegetable oils, fats, and corn flour, and is also made from the agricultural byproducts, usually fossil fuel derived from petroleum or natural gas. Bioplastic are mainly used in the packaging because it controls the pollution in the environment. Bioplastics are classified into variety of types. The most common of all is PHA (Polyhydroxyalkanoates), which is a carbon and/or energy saving substance in various microorganisms in the presence of low nutrient components¹⁴. By definition, the bacteria and other organisms are able to break or degrade the bioplastics¹⁵.

The biodegradable plastics are classified based on their origins as petrochemical-based or bio-based based¹⁶. The enzymatic action of microorganism on degrading bioplastics are monitored by the standardized test during which it undergoes a structured decomposition into methane, carbon dioxide and water as major components. Employment of new techniques in the production of bioplastics promotes a sustainable solution for the



reduction of plastic waste with food waste in the long-term. Hence, it is easy to replace the petroleum-based plastics by the bioplastics and could overcome the pollution and other environmental threat created by non degradable plastics.

3. Pomegranate Peel

The pomegranate peel comes under the family Lythraceae. The species is *Punica granatum*. It is a healthy and delicious shrub. It is used in cooking, baking, juices, soft drinks and food decorations¹⁷. The pomegranate has rich source of bioactive components and minerals¹⁸.

The *Punica granatum* has the composition of pectin-27%, hemicelluloses-10.8%, cellulose-26.2%, lignin-5.7%. On acid hydrolysis in pomegranate peel, the polysaccharides are converted into monosaccharides which can break down the lignin, cellulose and hemicellulose component that can make them a bioplastic.

4. Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) is a bluestrophytic hydrotophyte, a cosmopolitan aquatic weed. This creates dense invincible mats across the water surface, which limit access to man, animals and machinery. Also, navigation and fishing are prohibited, and irrigation and drainage systems are impeded. It can tolerate environmental parameters such as temperature, illumination, pH, salinity, wind, current and drought¹⁹.

Cellulose, lignin and hemicellulose content in the water hyacinth are converted into Polyhydroxybutyrate (PHB). PHB is the polymer which is employed in making biodegradable plastic. Currently, synthetic polymers and plastics are derived from petroleum-based raw material like polypropylene and polyvinyl chloride which cannot be decayed easily. Plastic fertilizer created using PHB. Furthermore, generating PHB from natural resources can reduce harmful gas emissions and cost. PHB can occur in the presence of inorganic and organic nitrogen sources.

5. Potato

Potatoes are world's major energy sources. Around 150 different types of potatoes are cultivated in a short period of time and could adopt to any type of climatic conditions. They serve as an important nutrient source for human and animal across the globe²⁰. The waste potatoes and peels of it are majorly used as animal feed or biofuel production, however, they contain large amount of valuable compounds that make possible to use them in pharmaceutical applications, when they take into account bio-economic principles. Bioeconomy is a science-based method that analyzes the rational and effective use of local resources to create new, value-added products for the existing market²¹.

The starch content in the potatoes ranges from 13 -23 % which makes it ideal for some plastics production. The starch-based plastic polymer can be further processed along with other biodegradable components such as the

Poly(lactic acids (PLA) or Polycaprolactone (PCL) in a conventional plastic extruder machine to produce the desired plastic product. There are some parameters such as the temperature or blending ratio that need to be respected during the processing of the biodegradable plastic. According to a study, the ratio of starch polymer to the additive can be 70:30 on a dry basis²². Also this study shows that the properties of the final product will depend on the type of starch used for the production of the plastic product.

6. Orange Peel

The peels of the citrus fruits normally contain carbohydrates which can be employed for production of commercially important industrial biomolecules. Sweet orange (*Citrus sinensis*) most commonly cultivated tropical fruit belongs to the Rutaceae family²³. Careless discharge of peels while unprocessed often causes serious environmental issues²⁴.

Pectin, starch, lignin, cellulose, and hemicelluloses, make these lignocellulosic feedstocks interesting and promising for the production of bioplastic films. These compounds are obtained from various fruits and vegetables. The production of biofilms from variety of polysaccharide residue feedstock is the latest trend in the bioplastics production²⁵. In bioplastics made from polysaccharides the introduction of intermolecular bonding is often necessary²⁶.

7. Polyhydroxyalkanoates

The Polyhydroxyalkanoates were first described by French scientist Lemoigne²⁷. This was the case in the storage polymer in prototype cells. Many bacteria are intracellular compounds, energy storage compounds and carbon²⁸. Polyhydroxyalkanoates (PHA) is a family of polyesters that are synthesized and accumulated into a wide range of microorganisms. It is the only plastic produced exclusively by microorganisms, and is completely reduced to harmless compounds²⁹. It is composed of 3-hydroxy acid monomer units and small number of ground particles per cell³⁰. PHA was in two forms, high molecular weight linear polyesters, which are present in gram-negative and gram-positive bacteria at 60000 daltons. The second is the simplest monomer building block form of PHA called polyhydroxybutyrate (PHB) which has a lower molecular weight of 15000 daltons. It is biodegradable and highly flammable by combustion, it also replaces petroleum polymers, and is involved in pharmaceutical, packaging and coating materials, manufactured for the future, woven materials, pharmaceuticals in surgical treatment, surgery, pharmacology and tissue engineering.

8. PHA PRODUCING MICROORGANISM

Alcaligenes latus, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus megaterium R11*, *Serratia ureilytic*, *Pseudomonas aeruginosa*, *Comamonas testosteroni*, *Pseudomonas guzezei*, *Enterococcus sp*, *Brevundimonas sp*, *Bacillus aryabhatai*, *Bacillus thuringiensis*, *Bacillus mycodies*,



Bacillus licheniformis, *Bacillus megaterium uyuni*, *Bacillus subtilis* are the organisms which produce polyhydroxyalkanoates.

9.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is gram-negative, rod shaped bacteria, citrate catalase and oxidase positive. It is often found in soil, skin flora, water and vegetation. It was discovered by Carle Gessard a chemist and bacteriologist in the year 1882 at Paris. With their water-soluble pigment, the bacterial culture turned to bluish-green when exposed to ultraviolet light. It grows in the laboratory on the agar plate, broth, which has a distinctive smell like the unpleasant smell of grapes, corn tortilla. The genome has a large circular chromosome, ranging from 5.5 to 6 megabyte that carries between 5500 and 6000 open reading frames.

It grows well in culture at 37°C and tolerates temperature up to 42°C, which can withstand environmental stresses. This organism generates extensive colonization and accumulates in a set of biofilms. *Pseudomonas aeruginosa* degrades naturally and completely into carbon dioxide and water under aerobic conditions and into methane under anaerobic condition³¹. Under the conditions of excess of the carbon source and controlling nutrients such as nitrogen, phosphate and oxygen, many types of microorganisms may naturally accumulate PHB as internal energy storage products.

Production of PHA in fed batch and the continuous culture of *P.aeruginosa* has been investigated to achieve high concentration of PHA and high productivity. *P.aeruginosa* can accumulate mixture of PHB and PHAs when grown on glucose and octanoic acid. The intracellular formation of PHA in *P.aeruginosa* in media grown contain *n*-alkanoic acids, by carbon formate to decanoate. However, the PHA formation was only observed for hexanoate and more number of *n*-alkanoic acids. It was found that monomer structure of the PHAs depends on the structure of the corresponding fatty acids which are derived from carbon source. The percentage of monomer content in PHA is adjusted by regulating the rates of fatty acids present in the growth substrates. Therefore, PHA structures are created by applying different proportions of structured related fatty acids and varying ratios of fatty acids³².

9.2 *Bacillus subtilis*

Bacillus subtilis is gram-positive found in humans and ruminants in the soil and gastrointestinal tract. It is also called as grass bacillus or hay bacillus. It was named *Vibrio subtilis* by Christian Gottfried Ehrenberg and renamed *Bacillus subtilis* in 1872 by Ferdinand Cohn. The bacteria were rod-shaped, about 4 -10 micrometres long and 0.25-1.0 micrometres in diameter. It was resistant to unfavourable environmental conditions such as extreme pH, radiation, drought, solvent and salinity. It is used as a soil inoculants in horticulture and agriculture, it is also used as an indicator organism during gas sterilization.

A number of *Bacillus* species are employed for the PHA production process but not all can accumulate PHA in their cells³³. *Bacillus* species are advantage over other PHA producing bacteria due to the abundance even in higher growth rates, extreme ecological conditions and absence of lipopolysaccharides. Additionally, *B.subtilis* is a GRAS (Generally Recognized as Safe) organism by the Food and Drug Administration³⁴. However, the well-characterized *B.subtilis* does not possess the genes for biosynthesis and depolymerization of PHA³⁵. The wide range of assimilable carbon sources and ability to secrete a number of hydrolytic enzymes for some *Bacillus* strains is also constructive for varied PHA production from economical agro-industrial and other waste materials³⁶.

9.3 *Alcaligenes latus*

Alcaligenes is Gram-negative, aerobic and rod-shaped bacteria employed for the non- standard amino acid production and also for polyhydroxybutyrate. The species is Amphitrichous flagella and is rarely invariant. It is a type of non-bacterial (*Alcaligenaceae* family). Also, some strains of *Alcaligenes* is capable of anaerobic respiration but must be present in the presence of nitrite or nitrate; Otherwise, their metabolism is never fermentable and respiratory. The genus cannot use carbohydrates. Structures of *Alcaligenes* (such as *A.faecalis*) is often found in the rotting materials, dairy products, intestinal tract of vertebrates, soil and water. They can be isolated from human respiratory and gastrointestinal tract and injuries in hospitalized patients.

Alcaligenes latus utilizes simple carbon sources and nitrogen sources as an intracellular metabolite for the production of PHA and a backup carbon source when an unfavourable environment is met. Microbial PHA is very biocompatible, biodegradable thermoplastic when compared to traditional thermoplastics. PHA can be synthesized intracellularly from sugars and fatty acids by the condensation of D-3-hydroxybutyryl-CoA, formed from acetyl-CoA via acetoacetyl CoA and butyryl-CoA, respectively³⁷.

9.4 *Micrococcus*

Micrococcus is a type of bacteria in *Micrococci* family. *Micrococcus* occur in a variety of environments, including dust, soil and water. Micrococci is gram positive spherical cell that are about 0.5 to 3 micrometres in diameter and are commonly found in tetrads. They are oxidase-positive, catalase-positive, nitrate negative and indole negative. *Micrococcus* has significant cell wall, which can make up to 50% of cell mass.

Predominately it is found that Gram positive are capable of commercial production of PHA when compared with Gram negative bacteria. Gram-positive bacteria is more beneficial than the Gram-negative bacteria because of the absence of lipopolysaccharides (LPS) which make PHA an excellent source of biological medicine. However, several other Gram-positive bacteria known to harvest lipidated macroamphiphiles which includes lipoteichoic acids (LTA)

and lipoglycans, which may have immunogenic property similar to LPS. Further examination will required to verify if there is any other lipidated macro amphiphiles in PHA-producing bacteria and Gram-positive. In vivo or in-vitro evaluation studies of Gram positive bacteria are necessary to evaluate the PHA producing bioplastics suitability for biomedical applications ³⁸.

9. Qualitative Confirmation of Bioplastic Production

The bioplastics produced by various organisms can be qualitative confirmed by many methods before it gets validated by GCMs and FTIR. The important method is

Sudan Blue confirmatory method. In this, method the bacterial culture is smeared with Sudan black B solution and counterstained with safranin dye. The appearance of bark blue under oil immersion microscope confirms the presence of PHA in the culture ³⁹.

Another important method with nitric acid, in this method the sample will be treated with nitric acid and distilled water. If it results in the appearance in blue colour results in presence of PHA. The last method is mixing of centrifuged cell suspension with hydrochloric acid and chloroform and obtaining a sharp peak at 240nm ^{40,41}.

Table 1: PHA producing microorganisms with different substrates

Substrate	Microorganisms	Temperature	pH	Type of Fermentation	Reference
Pomegranate	<i>Pseudomonas aeruginosa</i>	34°C	6	Aerobic	Ezeoha <i>et al.</i> , (2013)
	<i>Bacillus subtilis</i>	37°C	7	Anaerobic	Ezeoha <i>et al.</i> , (2013)
Orange peel	<i>Bacillus subtilis</i>	37°C	6	Anaerobic	Mridul Umesh <i>et al.</i> , (2017)
Potato	<i>Bacillus subtilis</i>	37°C	7	Anaerobic	Vivita priedniece <i>et al.</i> , (2017)
	<i>Alcaligenes latus</i>	37°C	6	Aerobic	Bingqing Wang <i>et al.</i> , (2013)
	<i>Micrococcus</i>	37°C	7	Aerobic	Priya Trivedi <i>et al.</i> , (2016)
Water Hyacinth	<i>Pseudomonas aeruginosa</i>	34°C	6	Aerobic	K Preethi <i>et al.</i> , (2015)
	<i>Bacillus subtilis</i>	37°C	7	Anaerobic	K Preethi <i>et al.</i> , (2015)

10. Market Demand

The utilization and the demand of bioplastics in the market continues to grows linearly every year. At present, 335 million tonnes of bioplastics are produced annually. According to latest market data, compiled by European Bioplastics in partnership with research firm Nova-Institute, it is expected to grow from about 2.11 million tonnes in 2018 to 2.62 million tonnes by 2023.

The increase in demands of bioplastics use across all market segments of sustainable products for consumers and brands, as well as reducing environmental awareness and reliance on fossil resources. Continued developments and innovations in the bioplastic industry in new materials with the improved properties and new functionality. Nowadays, there is an bioplastic substitute to every orthodox material of plastic and its use. Depending on the material, bioplastics have same properties compared to conventional plastics and offer supplementary paybacks like reduced carbon footprint or added waste management assessments such as industrial fertilizers ⁴². Bioplastics are used in a large sectors of markets in packaging, catering products, etc., Packaging is the major utility sector for bioplastics, accounting for nearly 65 per cent of the complete bioplastic market in 2018.

11. Application

Bioplastics are used in a large number of markets in packaging, catering products, consumer electronics, automotive, agriculture/horticulture and toys from textiles

and many other sectors ⁴². Packaging is the largest utility sector for bioplastics, accounting for nearly 65 per cent (1.2 million tonnes) of the total bioplastic market in 2018.

CONCLUSION

This paper has covered various substrates and microbes involved in the production of bioplastics. In summary, recycling is one policy for end-of-life waste treatment of plastic products. This makes sagacity for the environmentally and economically increasing and current trends to validate a significant increase in the rate of recycling and reprocessing of plastic waste. This inclinations are like to continue, but there may be momentous social and economic behavioural issues related to technical factors and collection of recyclable waste, and some significant challenges to the replacement of non-contaminated materials.

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