

# Polyhydroxybutyrate Produce by Lignocellulose

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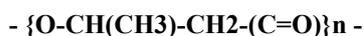
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## I. INTRODUCTION

The increasing worldwide concern of pollution is major threat and plastic is one of the major sources of the invasive pollution and pollution caused by plastic is one of the most widespread problems which are affecting our environment (Kumar,R,Sharma.2018). Plastic causes various pollution such as land pollution, air pollution and water pollution, and also cause different types of threat to human health, ocean health, food safety and quality. The burning of plastic and other waste product releases the harmful materials which cab be heavy metals, persistent organic pollutants, and release other chemicals into the air. These chemicals are also linked to the development of asthma, cancer and other diseases, but still it are being use in the industries because it is lighter in weight, show high resistance, low density, stability and flexibility and also is a poor conductor of heat and electricity. Based on the multiple beneficial properties of plastics they are most widely produced, as plastics show countless advantages but they also bring a lot of environmental problem and health hazards. Particularly, micro- plastics have direct effects on food products and also enter the human body. It is assumed that these micro-particles can possibly forward from the intestine to the blood and the lymphatic system and to various organs. It is also observed that one third of the plastic waste undergoes recycling, the left over end up in landfills polluting the environment. Most of the plastics manufactures are only single use and thus its increasing production leads to degradation of fossil fuels. But the excessive use of plastic is causing more risk. To reduce the increasing risk of environmental pollution an alternative should be use which show somewhat property similar to that of plastic or can use bio plastics. Bioplastics are defined as polymers of biomass mostly carbon based compounds and are absolute eco-friendly plastics. The Polyhydroxyalkanoates (PHAs) is biodegradable plastics and among which Polyhydroxybutyrate (PHB) the most well-known and are environment friendly materials which have property similar to plastics and belong to class of alkanooates. PHB are the biosynthetic and biodegradable materials which cause no harm to the environment and it can be biodegradable into organic waste (Li et al.2016, Umesh et al.2018) PHB can be broken down by living organisms, like bacteria as it is biodegradable in nature (Sirohi et al. 2020b). When PHB and the materials created by PHB is thrown out and it will naturally degrade without causing pollution. Bacillus megaterium was the first to generate Polyhydroxybutyrate.This was the first bioplastics made from bacteria. The chemical formula of PHB is show below



PHB are the recyclable polyester which have optical activity and piezoelectricity and can be refer as a good barrier. Properties of PHB are as it is partially crystalline in nature and have high melting temperature and crystallinity factor is also high. The nature of PHB is that they are stiff and brittle and degree of brittleness is dependent on crystallinity and also on the glass temperature and microstructure. The nature of brittleness increases at the room temperature. PHB does not contain any chain branching like other synthetic polymer. The important point of PHB is that they do not liberate any harmful residue into the environment. There are many PHB whose property is somewhat similar to polypropylene and polyethylene. The production of PHB biopolymer includes the renewable sources and sustainable sources such as food waste, agriculture waste and any other waste.

## II. REVIEW OF LITERATURE

PHB is a product of microbial secondary metabolism synthesized in the cells of microorganisms. PHB can be constructed by combining different substrates and growing them under various working conditions, which including aerobic and anaerobic, temperature, pH, and submerged or solid state. Renewable resources (such as starch, cellulose, and sucrose), waste materials (lignocellulose, molasses, whey, and glycerol), and chemicals such as propionic acid are used to make PHB. The use of renewable resources and waste materials as substrates for the synthesis of PHB is advantageous. When PHB is produced at industrial scale, there are currently a number of constraints. PHB production

faces a number of problems, including high production costs, limited yield, sensitivity to degradation, and technological complications. Lignocellulose material can be used to reduce manufacturing costs and achieve economic viability. But even though lignocellulose has a high fermentability, producing PHA from it can be challenging. 30-50 percent cellulose, 20-50 percent hemicellulose, and 15-35 percent lignin compensate lignocellulosic biomass. Although cellulosic biomass is inedible, its use does not compete with food or animal feed production (Alvira et al., 2010). However, one of the primary weaknesses of using lignocellulose raw materials for the production of PHAs is that lignocellulose is unmanageable. To obtain sugar feed stocks that can be digested by bacteria to produce bioplastics, pretreatment or hydrolysis procedures are necessary. *Pseudomonas pseudoava* was able to grow on the hemicellulose component of poplar wood until it reached a concentration of 30% hydrolysate, yielding 17 to 22% PHB (Munoz et al. 2008). Lignocellulose contains sugars with aromatic bonds and chirality, as well as phenols, and the oxygen to carbon rate is much higher than petroleum. Even though compared to petroleum, the conversion of oxygen to carbon is substantially higher, substituting transportation fuels would necessitate twice the amount of biomass as crude oil. The carbon and oxygen linkages, as well as chemical variation, may be required qualities in the synthesis of chemicals and polymers, such as acrylic acid.

The growing biofuels and bio based products have sparked a surge of interest and lead to the enlargement of a number of useful lignocellulose conversion methods. The top-down method modifies the PHB production infrastructure that is already in place. The sole difference between these techniques is the lignocellulosic materials and lignin fractions were recovered. To begin, lignocellulose pretreatments were created to improve cellulose to glucose enzymatic conversion. Pretreatments boost cellulose accessibility, decrease crystallinity, and eliminate inhibitors in lignin and hemicellulose, which are both feedstocks for biobased products in integrated procedures. Fractionation techniques are pretreatment procedures that aid in the separation of lignocellulose components. Concentrated phosphoric acid as a cellulose solvent-based lignocellulose fractionation is a three-step technique that beginning with an organic and is proceeded by water to achieve complete separation of hemicelluloses. Lignocellulose is divided into three portions as a finished product. Ionic solutions, which can dissolve both cellulose and lignin, are another option. 1-butyl-3-methylimidazolium chloride is the universal ionic liquid for cellulose solubilization, whereas lignin is commonly dissolved in 1-ethyl-3-methylimidazolium acetate. Bacteria can create PHB under unfavourable growth circumstances. Some bacteria can create PHB at a rate of up to 90% of dry cells. For industrial PHB manufacturing, bacteria such as *Pseudomonas putida*, Recombinant *E.coli*, and *Bacillus* spp. were employed. PHB is made from an important carbon metabolite derived from acetyl-CoA via a series of three enzymatic reactions

1. Beta-ketothiolase catalyses the reversible condensation of two acetyl CoA molecules to create acetoacetyl-CoA.
2. An acetoacetyl-CoA reductase converts acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA.
3. The enzyme PHB synthase catalyses the polymerization of (R)-3-hydroxybutyryl-CoA to start generating PHB. (Shrivastav et al.2013, Batista et al.2018)

PHB is produced by the interaction of triple enzymes on bacteria that produce acetyl-CoA. The 3-ethiolase catalyses the formation of a carbon-carbon bond between two acetyl-CoA molecules, meanwhile the acetoacetyl-CoA reductase (NADPH dependent) catalyses the conversion of acetoacetyl-CoA to R-3-hydroxybutyryl CoA. The polymerization of R-3-hydroxybutyryl-CoA to create PHB is catalysed by the enzyme PHB synthase (Gerngross et al., 1993). A partially crystal-like PHB polymer comparable to polypropylene and polyethylene is formed by the biosynthetic reaction of acetyl-CoA. PHB can be obtained using a variety of fermentation procedures, including discontinuous processes like batch culture, fed-batch culture, and recurrent fed-batch culture.

For the production of PHB materials, the batch culture technique is a straightforward discontinuous procedure. It's one of the least effective approaches. The physiological preconditions of the production strain limit the concentration of nitrogen and carbon sources at the start of batch fermentation. Because batch culture procedures have low productivity, a simple repeating batch approach was recently examined to improve volumetric productivity, and it successfully increased and demonstrated a considerable benefit over simple batch processes in the manufacture of PHB resources.

A good feeding strategy is adopted during the fed-batch procedure. The fundamental issue with fed-batch fermentation is maintaining control over the feeding substrate concentration in order to achieve an appropriate range of limiting and inhibiting values. The substrate feeding method used for successful PHB synthesis is critical (Xia et al., 2017; Li

and Wilkins, 2020, 2021; Nygaard et al., 2021). Continuous fermentation methods are known to operate in a steady state, which means that all of the variables that can affect the process, including as pH, nutrient supply, and product concentration, are kept constant. Many businesses that rely on microbial production, such as the beverage industry, are familiar with these methods.

When critical nutrients are limited or depleted, bacteria undergo a physiological stress response that results in PHB production. To make PHB, the bacteria must first go through a cell growth phase in which they are supplied vital nutrients to the level required for PHB production, and then the nutrients must be depleted after a particular amount of time to activate PHB production.

The continuous fermentation process aids in the production of high yields of PHB materials, including those arising from cultures with high specific growth rates, but PHB production has been limited due to the continuous process's susceptibility to microbial contamination and productivity disruptions, resulting in economic losses. Batch culture requires bigger bioreactor facilities to achieve the same output per time due to higher volumetric productivity in continuous processes, but continuous production leads to cheaper investment costs of smaller operation facilities. As a result, continuous cultivation for PHB biosynthesis is clearly more efficient in terms of productivity and product quality, resulting in greater downstream processing efficiency and improved performance attributes of PHB-based bioplastics.

### III. METHODOLOGY

The processing of PHB begins with the use of sunlight. The metabolic processes for producing PHA from carbohydrates are inextricably related to microbes' fundamental catabolism and anabolism. PHB production begins with sugar transfer into the cell, followed by catabolic conversion to pyruvate, then to acetyl-Coenzyme A, and ultimately to PHAs in a three-step process. PHB can be made from glucose or agricultural wastes like lignocellulose or molasses as a starting material. Sugars provide energy, carbon, and electrons via creating ATP, NADH, or NADPH, which is followed by the synthesis of pyruvate. Embden-Meyerhof-Parnas (EMP), Entner-Dodoroff (ED), and the Pentose-Phosphate Shunt are three enzymes that catabolize lignocellulosic sugars to poly(3-hydroxybutyrate) (PPS).

D-mannose and D-galactose are carbon-6 sugars that can enter the same pathways as D-glucose following conversion, whereas D-xylose and L-arabinose are carbon-5 sugars that are transformed with the PPS. EMP, ED, or PPS catabolize all of the major sugars in lignocellulose hydrolysates. To enter the catabolic pathways, mannose is phosphorylated to mannose 6-phosphate and then directly isomerized to fructose 6-phosphate. Galactose is transformed to UDP-galactose, which then enters the catabolic route via glucose 6-phosphate. The pentoses xylose and arabinose, unlike hexoses, can only enter catabolism through the pentose-phosphate route. Reduced pyruvate and thus lower PHA yields result from the pentose conversion. PHB biosynthesis follows a broad route.

1. For pre-fermentation, selected bacteria are utilised, and the cells are cultivated at 30°C in a stream of air in a mineral medium containing glucose as a carbon source. After 24 hours, the cells had multiplied to a cell density of around 20g/l (Sirohi et al., 2021b).
2. The cells continue to proliferate and achieve a cell density of 100 g/l after 40-80 hours. PHB is found in cells as distinct granules with sizes ranging from (0.3-1µm). The cell has an 80 percent dry mass in the form of PHB at the end of the fermentation phase. We may estimate that 1 kg of PHB consumes around 2.8 kg of sugar.
3. There are two primary methods for isolation.
  - Mechanical loads damage the cell walls, and the polymer is subsequently extracted using chloroform or another solvent such as methyl chloride, 1,2-dichloroethane, pyridine, or propylene carbonate. Centrifugation and solvent filtration are required to separate the cell remnants.
  - At 37°C, enzymes degrade the cell wall in an enzymatic process.

#### 3.1 Expected Outcome and Significance of Study

Plastic is amongst the most noteworthy pollutant sources in the environment. Plastic has become an environmental disaster, most of the plastic is made from petroleum and are non-renewable that destroy the ecosystems. The generation of PHA from lignocellulose is still in the early stages of study, but the results are promising. The majority of research employed lignocellulose to hydrolyze cellulose and hemicellulose to carbon six or carbon five sugar combinations with

excellent profits. To profitably undergo hydrolysis lignocellulosic biomass into carbon six or carbon five sugar combinations. Though conversion of lignocellulosic materials hydrolysate to PHB yields are reduced, with *Burkholderia* sp. F24, the greatest concentration thus far was 12.3 g/L. Xylose and arabinose, the primary sugars found in the amount of such hemicellulose substances hydrolysis process converted into biopolymer is lowered efficient metabolic pathway than glucose metabolism. Microbes that consume carbon five sugars more efficiently, whether it be through genetic alteration and separation of carbon five sweetened bacteria can still result in significant improvements. Whereas the formation from lignocellulosic biomass to PHB has been recognised, and, in contrast the progression from lignocellulosic biomass into there are still only a there are only a few concepts for integrated lignocellulose conversion processes

#### IV. CONCLUSION

The use of plastic in day to day life has increased due to its production and importance which create problem in the lengthy term, so there is requirement of suitable, economical, harmless alternative which is in huge demand. To replace these, biodegradable polymers came into the fame and are Polyhydroxybutyrate. Polyhydroxybutyrate (PHB) belong to the polyesters class and are biodegradable in nature. In this report we have studied those different-different characteristics of PHB and their benefits using them over plastic. By using PHB we can reduce the amount of pollution created by us, as PHB show property similar to that of plastic. The characteristics of PHB resemble with plastic so the product produce by PHB are also of similar feature. The PHB can be originated from renewable sources. The production of PHB is growing now a days into the market and the new developing fermentation process are implemented in many areas. The PHB is also having many problem which are being studied and trying to cover the problem so that PHB can replace plastic completely. Plant residues, such as domestic wastes, total 1.55 billion tonnes average annual, which is roughly five times the annual world plastic production. This lignocellulosic biomass accounts for about 44% of total annual plant production.

Around 69% of the total areas is cover by agricultural area which don't require fertilizer which will harm the soil and due to this fact grasses have great potential. As the production of PHB came into light and is growing rapidly and using cheap waste product are under trial and out of which lignocellulose product are good source. PHB productions by lignocellulose are very effective. Lignocellulose contains plant residue out of which wood is the good source of carbon. The lignocellulose fragments of hemicellulose portion have been the most feasible and effective feedstock for PHB synthesis because they are underutilised and can be integrated with bioethanol production from the cellulose share. PHB can be introduced as a co-product to fractionation operations to separate cellulose, hemicellulose, and lignin.

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