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Polyhydroxybutyrate As A Biopolymer

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I. INTRODUCTION

This project is about the use of biopolymer (PHB) as an alternative form in place of non-biodegradable plastic, many types of research have been done on this polymer which shows it sharesa similar application as that of conventional plastic and also are biodegradable and biocompatible. As synthetic polymers are cheap and inexpensive but are non-biodegradable and release toxicity that is hazardous for biomass so there is a need to switch on another alternative to reduce the dependence on conventional polymers.

In recent years, the increasing population that resulted in urbanization and industrialization has led to a surge in petroleum-based plastics consumption. Due to its cheap availability and durability, there has been a great demand in every field. The continuous increase in the use of petrochemical plastic is the cause of various environmental pollution as it is non-biodegradable and take thousandof years to break down so produce toxins if it is disposed of in soil and water, even if they burnt that will increase the CO2 concentration in the atmosphere.

There has been neededfor a biopolymer that can compete and reduce the dependency on hazardous plastics. Researchers have been looking for an alternative that would be eco-friendly and sustainable in nature as well as meet the current demand. Polyhydroxybutyrate (PHB) being biocompatible as well as biodegradable can be a better option to switch petroleum-based plastic as it possesses similar physical properties to that of propylene.

PHB is a biopolymer that is a member of the PHA family.PHA are of three types based on the number of monomers present*(Madison and Huisman 1999)*; the short-chain length consists of 3-5 units of carbon, medium chain length consists of 6-14 units of carbon, and the long-chain length consists of more than 15 units of carbon atoms*(Anderson and Dawes 1990)*. The nature of PHB is brittle and crystalline as well as high melting and degradation point due to the presence of double bond that leads to the chemical variation and contrast structure*(Colin et al, 2012)*.To improve its properties we have to blend it with other natural plasticizers such as (glycerol, soybean oil, triethyl citrate, salicylic ester) to reduce its brittleness and improve lowering temperature

PHB can be obtained in the form of carbon energy sources from many micro-organisms such as bacteria, fungi, yeast, algae, eubacteria, cyanobacteria, archaebacteria. PHB can be produced both in excess carbon source and limited source of nitrogen, sulfur, oxygen*(Ceyhan and Ozdemir 2011)*.Manybacterial strains such as bacillus and pseudomonas contribute toa large amount of accumulation PHB in nutrient-limited condition

Another limitation of PHB is expensive synthesis and production cost and also have less elasticity as well as poor mechanical properties with low nucleation density that resulted in its restrained use, this can be solved by using natural and biobased raw materials such as plant pulp, agriculture waste, an industrial by-product, whey, molasse, this low-cost renewable resources ultimately leads to its large- scale production.

PHB possess a wide range of application because of its bio-sustainability and non-toxic nature examples; biomedical fields, food packaging, biofilms, nanocomposite coating, tissue engineering. In food packaging industries PHB is blended with nanoparticles to replace PP (polypropylene) and PE (polyethylene) in many common products such as shopping bags, cutlery, cups, containers. Still, there is a long way to go as despite being bio sustainable and biocompatible its annual production is around 30,000 tons *(Maximilian L 2017)*than that of PP production which is around 65 million tons.

They are used to develop medical equipment such as surgical staples, screws, plates, pins, sutures materials, etc*(Kavitha 2018)*. Using agriculture and industrial waste as a source of PHB production can be turned out cheap and inexpensive that will somehow reduce its production cost*(Kaur. et al 2017)*. Many fruits and vegetable waste that have renewable sources for cellulose, lignocellulose, etc can be utilized in PHB production*(Maraveas 2020)*.

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II. LITERATURE REVIEW

PHB can be produced from bacteria, algae and marine microbes they are rich in carbon and energy sources. Nowadays genetic engineering is also been used to produce PHB as a biological extraction method. As its production and purification cost is high we can focus on renewable sources and use low cost-effective raw materials for PHB production such as starch, agriculture residue, industrial waste, and also from plant sources. PHB hasa wide range of applications other than plastic such as in packaging, agriculture, tissue engineering, medical fields, biofilms.

Bacterial strain:

- Actinomycetes: It is a gram-positive unicellular bacteria widely used in the synthesis of metabolites. Streptomyces are reported for PHA production in granular form for antibioticsThere are chances that it can be used for PHB production which is capable of replacing petroleum-based plastics. Till now only 12 different strains have been identified in genus streptomyces. In the presence of excess carbon source and nitrogen as supplements, Streptomyces griseorubiginosusaccumulates PHB up to 9.5% in mycelial dry mass *(Manan et al. 1999).*
- Halophilic bacteria: It is a type of marine bacteria present in a high saline environment and requires salt concentration from 5% to 10% (w/v) for cell growth *(Oren, 2008).* Other Halophilics bacteria that are capable of synthesizing PHA are Haloferaxmediterranei, Vibrio spp., V. nereis, V. natriegens, and V. harveyi (*Kavitha et al. 2018).* They could accumulate PHB with high molecular weight in various carbon sources; such as glucose, xylose, sucrose, sodium acetate, and butyric acid having dry cell mass up to 81% *(Quillaguaman, 2006).* Haloferaxmediterraneiis capable to accumulates PHB from 60 to 65% in cell dry weight at 25% (w/v) salt concentration in phosphate limited condition and excess carbon sources *(Garcia and Rodriguez-Valera, 1990).*
- Pseudomonas aeruginosa: It is gram-negative bacteria completely breakdown into carbon dioxide and water in aerobic conditions and to methane to anaerobic conditions *(Yoshiharu et al. 1992).* It accumulates PHB at excess carbon source and nitrogen, phosphate, and oxygen and limited condition *(Chozhavendhan et al. 2020).*
- Cyanobacteria: It is gram-negative photosynthetic prokaryotes found in marine bodies and can accumulate PHB up to 37% dry cell weight with CO2 as a carbon source *(Kamaravamanesh et al. 2018).* Using genetic engineering in cyanobacteria can increase PHB production. Moreover, the consumption of cyanobacteria at the industry level to synthesize PHB can be bio-sustainable and eco-friendly *(Brandl et al. 1990).* Synechosystissp PCC6803 accumulates high amount of PHB up to 38% in dry cell weight *(Arun et al. 2006).*
- Saccharomyces Cerevisiae: Unicellular fungus primarily used in the baking, brewing industry. Other than this it is also an important microorganism PHB production. It contains high sugar concentration as well as has high specific growth rate that resulted in the production of ethanol in presence of oxygen which further accumulates more PHB. The mixed concentration of glucose and ethanol on which S. cerevisiae is grown yields more PHB than that on a single substrate *(Kocharin and Nielsen 2013).*

Microorganism	Carbon source	PHA content (%w/v)
Alcaligenes latus	Glucose or Sucrose	> 75%
Bacillus Megaterium	Sugarcane molasses	42.1%
Haloferaxmediterranei	Sucrose, Oxalate, Lactate and Pyruvate	70%
Pseudomonas aeruginosa	Glucose	62.44%
C. Necator	Fructose	68%
Synechocystissp PCC6803	CO2	38%
Streptomyces griseorubiginosus	CO2	9.5%
Pseudomonas putida KT2440	Glucose	32.1%
E.coli	Glucose	75%
Ralstoniaeutropha	Glucose	80%

III. MEDICINE

PHB has biodegradable properties and can be disposed of easily so they are used to develop medical equipment such as surgical staples, screws, plates, pins, sutures materials, etc(Kavitha 2018). Because of having biocompatible and

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nontoxic nature it has been used to make drugs. Many antibiotics that are used as feed supplements for animals for their growth are also sourced from PHB. There has been the use of PHB as raw material for tablets(Ray and Chandra 2017).It is also been used in both in vivo and in vitro studies(Doyle et al. 1991).

3.1 Food Industry

PHB being biodegradable and biocompatible are used in food packaging. After blending it with another polymer to make it elastic and then it can use in packaging as it has brittle and rigid characters. Many types of research have been done to blend PHB with other natural polymers to make nanocomposites that can be used in making biofilms for packaging purposes. As food marketing is growing where it needs to use polymers that are non-conventional and also 100 percent biodegradable. PHB with properties like water-soluble, resistant to UV radiation, and non-toxic is the best fit for this application. As it is biodegradable and eco-friendly so there is no need to recycle which can reduce the time as well as the recycling cost(Darani and Bucci 2015).

3.2 Agriculture and Industrial waste

Using agro- and industrial waste as a source of PHB production can be turned out cheap and inexpensive that will somehow reduce its production cost(Kaur et al 2017). Many wastes such as whey, molasses have been used as carbon sources for PHB production. Sugarcane bagasse has been the major agro-based waste that is widely used for PHB production(Salgaonar and Braganca 2017).PHB that are produced from starch has great potential in biomedical, environmental fields and food packaging, etc(Lu and Xiau 2009).Wastewater that is released from different industries can be a good source for PHB production as it can be easily handled and also be cost-efficient. Many fruits and vegetable waste that have a renewable source for cellulose, lignocellulose, etc can be utilized in PHB production(Maraveas 2020).

IV. METHODOLOGY

First, collect the sample from a river where there is a chance of PHB producing bacteria. Then isolate the sample and culture it on an agar plate medium and incubate it at 37 °C for at least 24h.

The isolates can be screened by the Sudan black B method for 10 to 15 minutes and stained with the Bergy manual of determinative bacteriology for the detection of PHB accumulation.

We can prepare a medium for PHB production; glucose-1g, peptone-0.25g, yeast extract-0.25g, NaCl-0.01g, KH_2PO_4-0.05g, $MgSO_4$-0.002g, and at PH 7. The culture was then obtained at 8000 rpm for 15 minutes at 4°C.

4.1 Overview

PHB (Polyhydroxybutyrate) is a bioplastic polymer member of the PHAs (Polyhydroxyalkanoate) family. It can be obtained in the form of carbon energy sources from many micro-organisms such as bacteria, eubacteria, archaebacteria. PHB is a thermoplastic with high melting and degradation point. They are generally brittle and crystalline that can range from 50% to 80% reported by many studies. The presence of crystallinity hasan impact on its other properties such as its hardness, modulus, density, tacticity, transparency(Schneemeyer et.al 2003). It was the first isolated PHA's and is a linear chain polyester of D(-) 3- hydroxybutyric acid and can be accumulated as cell granule by different strains of gram-positive and gram-negative bacteria in nutrient-limited conditions (carbon excess)(Dawes et.al 1973).Thesemicrystalline isotactic stereoregular polymer plus 100% R configuration provides a high level of degradability to PHB(Noda et.al 1999).

Many studies revealed that PHB can be breakdown by micro-organisms to carbon dioxide and water in aerobic conditions(Sawada H 1998).It is generally has a similar feature as that of PP and PE (melting point and tensile strength) that made it competitive to petroleum-based plastic. Apart from biodegradability it also has other properties as resistance to UV radiation, water-insoluble, nontoxic, high degree of polymerization, piezoelectric, and optically active. PHB synthesis occurs by the reversible condensation of two molecules of acetyl CoA by the action of 3-ketothiolase which then produces R-3 hydroxybutyrate CoA which is polymerized to form PHBsynthase[Sharma, 2019].

Properties	PHB	PHBV	PHB48	PHBHX	PP
Melting temperature (°C)	177	145	150	127	176
Glass transition temperature (^{O}C)	2	-1	-7	-1	-10
Crystallinity (%)	60	56	54	34	50-70
Tensile strength (MPa)	43	20	26	21	38
Extension to break (%)	5	50	444	400	400

Table.2: Markl and et al. (2018). Cyanobacteria for PHB Bioplastics Production: A Review

V. CONCLUSION

PHB being eco-friendly have much potential to replace petroleum-based plastic. We can switch to PHB because of its sustainable and biodegradable nature. PHB is not much utilized commercially due to its high production cost. There not have been much work done on using natural raw materials such as plant-based cellular components like fibers, cellulose, ester. PHB can be produced from bacteria, algae and marine microbes they are rich in carbon and energy sources. Nowadays genetic engineering is also been used to produce PHB as a biological extraction method. As its production and purification cost is high so we can focus on renewable sources and use low cost-effective raw materials for PHB production such as starch, agriculture residue, industrial waste, and also from plant sources. PHB hasa wide range of applications other than plastic such as in packaging, agriculture, tissue engineering, medical fields, biofilms.

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