Bioplastic production from cyanobacteria and polyhydroxybutyrate accumulation - A review

Study of progress made on how bioplastic is derived from cyanobacteria and their PHB accumulation property

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***Abstract*: Bioplastic is described as a plastic made from renewable biological elements. Bioplastics made from renewable carbon resources contribute to our efforts to conserve scarce fossil resources as well as to protect from Petro-chemical derived plastic pollution. Carbon dioxide and water are the breakdown products of bioplastics. Bioplastics can be derived from many microorganisms, Starch, Cellulose, Protein, Organic, Aliphatic Polyesters etc. one of main bioplastic resource is- Polyhydroxybutyrate.**

Polyhydroxybutyrate, are potent bioplastic raw materials, which can be produced using cyanobacteria or blue-green algae as a host. The benefit of employing cyanobacteria to manufacture Polyhydroxybutyrate (PHB) over traditional fermentation methods is that it uses sugar or waste materials as feedstock. It is more sustainable because CO2 is the only carbon source and sunlight is the only source of energy utilized here. In 2021, researchers were able to get 36.1 percent of PHB dry cell weight generated in 7 days, which is close to 78 percent of PHB dry cell weight in heterotrophic microorganisms. The research shows that cyanobacterial PHB has a lot of promises in PHB production and by using different Agricultural Wastes as a low-cost substrate for it, like- Pre-treated orange, mango, banana, onion peels, and rice straw waste when employed as carbon sources, can lead the strain to collect up to 150-450 mg/L of PHB.

***Index Terms*: Biopolymer, Cyanobacteria, Polyhydroxybutyrate (PHB), PHB content.**

Abbreviations- Polyethylene (PE), Polyethylene Terephthalate (PET), Polyhydroxyalkanoates (PHA), Polyhydroxybutyrate (PHB), Polypropylene (PP) and Poly vinyl chloride (PVC)

1. INTRODUCTION

1.1 PLASTIC

Plastics are made up of polymers. A polymer is made up of multiple units called monomers, Polymers are molecular chains. Carbon, hydrogen, oxygen, and/or silicon are used to make each link of the chain. Monomers are hooked or polymerized together to form the chain. Plastics are made by heating petroleum and other materials under regulated circumstances and breaking them down into smaller molecules known as plastic monomers. Plastic resins are made up of several monomer combinations that have varied properties, such as strength or shaping ability.

Petrochemically generated polymers include polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), and polyethylene terephthalate (PET). Petro-chemical derived plastics are currently the main reasons of plastic pollution as they take thousands of years to degrade. Bioplastics takes less time to partially or completely degrade.

Bioplastic such as polyhydroxyalkanoates (PHA) are one example of biopolymers plastic that might replace bulk Petrochemical-derived plastics. PHA comprises around 150 different Monomers. Polyhydroxybutyrate is the most common form of PHA. In comparison to synthetic polymers, PHB offers a number of benefits.

PHB have better barrier quality than polyethylene and polypropylene as they are stiffer and less flexible. Another distinguishing feature of PHB is its- biodegradability within an acceptable timeframe when the material is used in biologically active settings such as soils, freshwater, and the presence of degrading microbes.[9]

They are sought-after eco-friendly due to their use of water, aerobic and anaerobic composting, and aerobic and anaerobic composting.

There are 2 types of Polymers-

Thermosets and thermoplastics, when heated a thermoset polymer solidifies or "sets" irreversibly. They're valuable because of their durability and strength, and they're also inexpensive.

Since 2004, the world has produced as much plastic as it did in the previous half-century, with an estimated total mass of virgin plastics of 8.3 billion tones, derived primarily from natural gas and crude oil, which are used as chemical feedstocks and fuel sources.

From a geographical standpoint, the five heaviest users of plastics are- China, Indonesia, the Philippines, Vietnam, and Sri Lanka, accounting for 56 percent of worldwide plastic trash.

Plastics have an important role in modern society, public health, and medicine. Human society relies on them but in return they are creating pollution. Hence, human should find some biodegradable substitutes as earliest as possible.

1.2 HISTORY OF BIOPLASTIC

In today's world, plastics are everywhere. Natural rubber was molded by human hands and polymerized into useful things in prehistoric Mesoamerica as early as 1600 B.C. The development of vulcanized rubber and polystyrene (PS) in 1839 sparked the commercialization of plastics. Bakelite, the first entirely synthetic polymer, was created in Belgium in 1907. Plastics manufacture began in the 1940s and has continued to grow since then. There are presently 20 main types of plastics, with an estimated global consumption of 245 million tones.[8]

The investigation for bioplastics has been started decades ago. In order to produce naturally derived bioplastics from polymers like PHB, PHA etc.

W.R. Grace (US) tested if bioplastics (PHA and PHB) may be made commercially from microorganisms and bacteria in the 1950s and 1960s.

1975 – A group of Japanese scientists discovers the biodegradable plastics idea. In pools holding waste water from a factory, they identified a microbe *(Flavobacterium*) that broke down nylon.

From year 1980s to till now many alternatives for bioplastics has been developed, still commercialization of bioplastics is huge problem due to due to a variety of problems, including high prices and instabilities in molecular weights (Mw) and structures, which result in thermo-mechanical instability. [13]

1.3 CHARACTERISTICS OF POLYHYDROXYBUTYRATE

Polyhydroxybutyrate (PHB) is a kind of PHA with a methyl R-group. It has a high crystallinity of more than 50% and a melting temperature of around 180 °C. The glass transition temperature is 50 °C, and the virgin material is brittle. Plasticizers, the most common of which is citrate ester, are used to overcome flaws like as brittleness and quick disintegration. The advantage of the polymerization reaction approach over microbial production is the ability to adjust the stereoregularity [18]

PHB is a biodegradable polyester with excellent optical activity, piezoelectricity, and barrier characteristics.it does not contain any catalyst residues. PHB is isotactic and does not include any chains.

Although it is not water soluble, it is completely biodegradable. PHB is permeable to O2, H2O, and CO2 at low concentrations. PHB has a disadvantage: it is expensive and thermally unstable during processing; hence it is not recommended.

Despite their current market share of around 2%, they expect double-digit growth in the near future. Statistics of increase examines the long-term viability of bioplastics. The annual output surpasses 300 million tonnes [14]. Polyhydroxyalkanoates are one of the examples of biopolymers, which might replace use of bulk Petrochemicals derived plastic in a variety of applications. PHA's most important representation is polyhydroxybutyrate (PHB), that can be produced from cyanobacteria.

Cyanobacteria are the only photosynthetic prokaryotes that can create oxygen, and they get their energy from photosynthesis. The word "cyanobacteria" derived from the Greek word "cyan," which means color blue. Cyanobacteria are also known as "blue-green bacteria or algae". Cyanobacteria can produce PHA photo-autotrophically, with the potential for CO2 recycling and bioplastics production. Cyanobacteria are attractive microorganisms for producing polyhydroxybutyrate in a cleaner circular setting because they can thrive on inorganic substrates like CO2 from industrial sources and nutrients via wastewaters. However, biotechnology production is difficult since it comprises a number of interconnected reactions that are influenced by external factors, making process optimization difficult. [16]

1.4 PHB, A LOW-COST BIOPLASTIC FOR MASS-MARKET APPLICATIONS

Petrochemically generated polymers include polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) are currently the main reasons of plastic pollution. To overcome this plastic pollution, we should focus on working with biopolymers like PHB as the barrier permeability of PHB is superior to that of both PE and PP, and it is also found to be more stiff and less flexible than PP. In addition, compared to PET and PVC, PHB has high barrier qualities. [7] Another important feature of PHB materials is their biodegradability, which occurs within a reasonable time frame when they come into contact with degrading microorganisms in biologically active environments like soils, fresh water, and aerobic and anaerobic composting, making them a sought-after environmentally friendly alternative to synthetic polymers.

PHB is a compound that is very similar to Polypropylene (PP) and has thus been proposed as a replacement for Polypropylene (PP).

Property	PHB	PP
Crystalline melting point (°C)	175	176
Crystallinity (%)	80	70
Molecular weight (Daltons)	5×10^5	2×10^5
Glass transition temperature (°C)	4	−10
Density (g/cm³)	1.250	0.905
Flexural modulus (GPa)	4.0	1.7
Tensile strength (MPa)	40	38
Extension to break (%)	6	400
Ultraviolet resistance	good	poor
Solvent resistance	poor	good

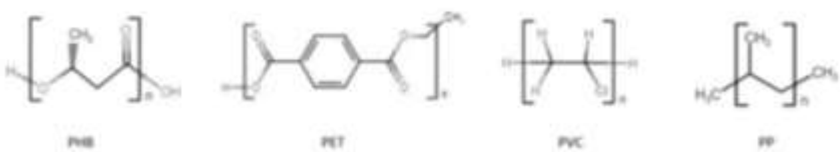
Table 1 Properties of PHB compared to those of PP (source: Kaplan DL,1998)

In Table1: *both the PHB and PP are being compared but it is easily noticeable that the PHB's low elongation and its brittleness are its major drawbacks. These can be avoided by employing alternative PHA, or copolymer mixes [7].*

Summary of mechanical properties of P3(HB) and petrochemical based (PP, PET, PE) and bio-based polymers (PLA).

Mechanical Property	P3HB	PP	PET	LDPE	HDPE	PLLA	PDLLA
Tensile modulus (GPa)	3–3.5	1.95	9.35	0.26–0.5	0.5–1.1	2.7–4.14	1–3.45
Tensile Strength (MPa)	20–40	31–45	62	30	30–40	15.5–150	27.6–50
Elongation at break (%)	5–10	50–145	230	200–600	500–700	20–30	1.5–20
Degree of Crystallinity (%)	50–60	42.6–58.1	7.97	25–50	60–80	13.94	3.5
Melting Temperature (°C)	165–175	160–169.1	260	115	135	170–200	amorphous
Glass Transition Temperature (°C)	5–9	−20—5	67–81	−130–100	−130–100	50–60	50–60

Table 2: *Properties of PHB compared to those of PP, PET and others (source- Utsunomia et al,2020, Amar B et al 2015, Horvath T et al 2018)*



Chemical structures of P3(HB) in comparison to commonly used petroleum-based polymers (polyethylene terephthalate (PET), polyvinylchloride (PVC), PP).

Figure 1 *property comparison (source Markl E., Grünbichler H., Lackner M. PHB—Bio Based and Biodegradable Replacement for PP: A Review. Nov. Tech. Nutr-. Food Sci. 2018; 2:206–209)*

Technology	Reasons and/or purpose	Methodology
High cell density fermentation	Achieve effective growth and cells recovery	Manipulation on quorum sensing and cell oxygen uptake mechanisms
Growth cells in low cost substrates or mixed substrates	Substrates contributed to over 60% of PHA cost	Screening targeted substrates utilizing bacteria able to produce high content PHA
Fast growing cells	Reduce fermentation duration and avoid microbial contamination	Minimizing bacterial genome, changing cell growth patterns
Fast growing CO_2 utilizing bacteria able to produce PHA	CO_2 is a free substrate	Manipulating the CO_2 uptake mechanism such as carboxysomes, etc.
Open (unsterile) and continuous fermentation process	To save sterilization energy, reduce fermentation complexity and improve process effectiveness	Screening for PHA producers able to grow fast in extreme environments such as high or low pH and temperature, high osmotic pressure, etc.
PHA synthesis induced by oxygen limitation	Oxygen is a limited factor in all high cell density growth	Place PHA synthesis operons behind microaerobic promotor
Ultrahigh PHA accumulation (over 95% PHA in cell dry weight)	To avoid expensive and complicated downstream PHA purification process	Manipulating the PHA synthesis mechanism and PHA synthases
Increase substrate (mostly carbon sources) to PHA conversion efficiency	Substrates contributed to over 60% of PHA cost	Removing pathways that consume substrates for non-PHA metabolisms, and/or reinforce PHA synthesis flux
Enlarging the PHA production cells	To allow more cellular space for PHA accumulation, this also allows easy cells recovery	Engineering the cell division patterns and/or cytoskeletons
Inducible cell flocculation	Allow easy biomass recovery after fermentation	Inducible expression of surface displaying adhesive proteins
Inducible cell lysis	Allow easy PHA granules recovery after biomass harvest	Inducible expression of cell lysis proteins
Cell disruption by PHA hyperproduction	Save the biomass harvest process	Manipulating the PHA synthesis mechanism and PHA synthases
Extracellular PHA production	Not limited by a small cellular space, also for easy PHA granule recovery	Need new PHA synthesis mechanisms
Large PHA granules	Allow easy recovery of PHA granules from lysis broth	Manipulating the formation of PHA granules associated proteins
A synthetic cell combining the above properties	Achieve up-stream and down-stream competitivenesses	An artificial cell with assembled functional DNA

***Table 3**: Technology to be developed to lower PHA production cost (source-Możejko-Ciesielska J, Kiewisz R. Bacterial polyhydroxyalkanoates: Still fabulous? Microbiological Research. 2016:271-282)*

PHB has a disadvantage, that it has a high production cost. In table 3 we can see number of methods that can make PHA manufacturing more cost-effective. [10]

A catalytic mechanism for PHB synthesis is an alternative for PHB production. Both the fermentation and catalytic processes produce a costly PHB product that is difficult to sell since it competes with low-cost commodities like PE and PP for market share

2. CURRENT STATUS OF KNOWLEDGE ON PHB SYNTHESIS BY CYANOBACTERIA

PHB can be produced by cyanobacteria as an intracellular energy and carbon storage molecule.

In 2016, Under photoautotrophic conditions the PHB concentration of 137 cyanobacterial strains comprising 88 species in 26 taxa were determined [4]. The presence of significant PHB content was strain-specific and had nothing to do with the genus. Only 134 of the 137 strains examined generated PHB, with *Calothrix scytonemicola* TISTR 8095 having the highest concentration (Thailand Institute of Scientific and Technological Research). In 44 days, this strain produced 356.6 mg/L PHB, with a PHB content of 25% of cell dry weight (cdw) and a total biomass of 1.4 g/L. Under nitrogen depletion, cells had a PHB concentration of 25%, but cells with nitrogen availability had a PHB content of just 0.4 percent. Only six of the 19 *Calothrix* strains examined generated more than 5% PHB of cdw. *Calothrix* has a number of benefits, including the relative simplicity with which the thick flocs of algae may be harvested, however cultivation of *Calothrix* is still in its early stages.

Same year, Ansari S, Fatma T also studied for PHB production, tested in 23 cyanobacterial strains (15 heterocystous and 8 non-heterocystous).

PHB levels were highest in *Nostoc muscorum* (6.44 percent w/w of dry cells). *Spirulina platensis* has the lowest PHB accumulation., which was confirmed using Sudan black B and Nile red A stains. The microscope Biomass was pre-treated with methanol: acetone: water: dimethylformamide. The extracted polymer was identified as PHB using FTIR, 1H NMR, and GC-MS.

When compared to their plant counterparts, cyanobacteria have various industrially significant advantages, including a quicker growth rate, stronger CO2 consumption, and more genetic engineering amenability.

In 2021, Utharn *et al* demonstrated that *Synechocystis sp*. PCC 6803 lacking adc1 gene (Δadc1) had the highest capacity to synthesize PHB under nutrient modified media. This Δadc1 mutant could grow as similar as *Synechocystis* wild type. Its highly accumulated PHB occurred up to 36.1 %w/DCW after adaptation in a nitrogen and phosphorus-deprived BG11 medium containing 0.4 %(w/v) acetate for 7 days. [17]

An overview of the generation of poly-hydroxybutyrates from cyanobacteria for bioplastic manufacture was published in 2013. Significant improvements have been made in the meanwhile. Troschl *et al* may be able to report 12.5 percent PHB cry well weight in 2018. [16]

In the same year, Kamravamanesh *et al* demonstrated that the cyanobacterium *Synechocystis sp*. PCC 6714 may create up to 37% dry cell weight of PHB using CO2 as the only technology to reduce PHA synthesis. [5]

This figure is much higher than any previous literature-reported value. UV light mutations were used to boost the PHB production of the strain. *Synechococcus sp*. MA19, a thermophilic cyanobacterium, had previously been reported to attain 27 percent dry cell weight PHB. [11]. The MA 19 was first isolated from a hot spring in Japan. However, neither the paper's authors nor additional researchers were able to collect a sample from that strain till 2018

Currently, Utharn's strain *Synechocystis sp*. PCC 6803 after Kamravamanesh's strain *Synechocystis sp*. PCC 6714 is considered as the cyanobacterium with the highest PHB content with 36.1 percent generated within 7 days.

3. CYANOBACTERIA PRODUCING PHB: PROSPECTS

The downstream processing of cyanobacteria, i.e., how to obtain the bioplastics material out of the cyanobacteria, is a big unresolved challenge. Despite the advantages of PHA over traditional plastics in terms of sustainability, lowering the costs connected with microbiological plastic manufacturing is required for fossil plastic to be viably substituted. Even when compared to other sustainable polymers, such as PLA, research and investments in the sector have reduced the cost of manufacture.

The primary roadblocks in the process are the carbon source utilized and the maintenance expenditures [15]. It can be solved by using agriculture wastes, in 2021 P R, Yashavanth et al demonstrated that According to their findings, agriculture waste (sugar peel waste) might be used directly as a low-cost nutrition source for biodegradable plastic manufacture. As a result, it may assist to tackle the problem of costly peel waste treatment as well as high biodegradable bioplastic manufacturing costs, as well as aid in the conservation of petroleum chemicals used in commercial plastic manufacture. [12]

The carbohydrate and protein content of agricultural waste material.

Agricultural Waste Material	Carbohydrate Content	Protein Content
Orange peel	82.7%	8.2%
Mango peel	46.8%	6.7%
Banana peel	50.9%	6.6%
Onion peel	20.5%	9.4%
Rice straw	46.5%	4.5%

***Table 4**: comparison among different agriculture waste and their biomolecule content (source- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8625221/table/microorganisms-09-02395-t005/?report=objectonly)*
These findings show that orange peel extract, followed by mango peel and banana peel extract, produced the most PHB, while onion peel and rice straw produced the least. [Rezk S.A. et al,2020]

Bacillus wiedmannii, isolated from cow dung in the Assiut governorate's shotb region, was able to efficiently use sugar fruit peel waste as a nutritional source for PHB synthesis. The isolate's optimal pH, temperature, and incubation duration for maximum PHB synthesis were 7, and 72 hours, respectively. [15]

Fixed carbon sources as a supplement increases the amount of carbon building blocks and energy available and speedup up the manufacturing. However, there are several drawbacks to autotrophic cultivation, such as (1) strict control of closed photobioreactors and (2) monoculture optimization in open pond culture. [1]

It was discovered that photo-mixotrophic production of PHB from Cyanobacteria has increases the titers by up to fivefold indicating that this has a bright future.

4. CONCLUSIONS

These studies provided an update on cyanobacterial PHB production, a technique that may be more sustainable than catalytic PHB synthesis, Carbon monoxide (CO) or sugar-based fermentation. It is discovered that PHB and its affiliates would be successful.

In many high-volume applications, compounds will progressively replace polypropylene. The production of PHB in cyanobacteria can be increased by genetic engineering; nevertheless, there are drawbacks. Like permit for large-scale cultivation in the works (Cost- and energy-efficient). In most countries, open growth systems will be tough to come by, thus technologies will be necessary. Avoiding genetic engineering appears to be the most commercially viable option right now. [6]

Despite the fact that PHB has a basic chemical composition, its granules are extremely complicated (Pseudo-organelles that require a variety of proteins to operate properly). PHB materials are stiff and brittle mechanically, with limited thermal stability and high crystallinity. PHB plastics have characteristics with petroleum polymers such as polypropylene (PP) and polyethylene (PE).

Renewable and sustainable feedstocks, such as food waste, are used to make PHB biopolymers. PHB is a prominent option as a replacement for synthetic polymers such as PP and PE due to its biocompatibility and proclivity for biodegradation when exposed to approved active biological conditions. PHB can have a variety of roles in an organism, ranging from molecule storage to stress tolerance and beyond. Despite the fact that the physiological function of cyanobacteria remains a mystery, the area of study is continually developing, allowing researchers to get a better understanding of its significance. The function of PHB in the control of intracellular redox equilibrium is one of the most promising possibilities. As a biotechnological application, this means that when high levels of reduction equivalents are sought, culture conditions that support high levels of reduction equivalents should be considered. Fundamental questions remain as such as the existence of a PHB depolymerase that has yet to be found. Because cyanobacteria are photoautotrophic, they might be used as a platform for carbon-neutral, long-term PHB production. As a result, more study in this area will be advantageous for industrial reference as well.

5. CONFLICTS OF INTEREST

The author has declared that they don't have any conflict of interest.

6. FUTURE PROSPECTUS

Biological researchers are interested in producing ecologically acceptable biopolymers from microorganisms such as cyanobacteria utilizing a low-cost substrate. The production of PHB bioplastic will help in making world plastic free and less polluted. It can save us from the problem of sewage water log, addition of microplastic in animal body, pollution and Biocontamination etc. cyanobacteria are photoautotrophic, they might be used as a platform for carbon-neutral, long-term PHB production

Because many nations throughout the world are experiencing food shortages, making bioplastics from trash rather than food is the greatest option. Food/ Agriculture wastes might be utilized to make bioplastics. The bioplastics made from potato peels entirely biodegraded in 28 days, according to this study, [2] and it was recommended that these bioplastics may be employed in the packaging business. The evolution of mechanical characteristics should be explored in order to apply it to various industrial applications. However, it was discovered that the commercial bioplastic did not biodegrade after 28 days. The use of bioplastics has expanded in recent years. As a result, the requirements for those labelled as "biodegradable" must be met to ensure their long-term viability.

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