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A review on Polyhydroxybutyrate (PHB): Production, Properties, Applications, and Environmental Implications

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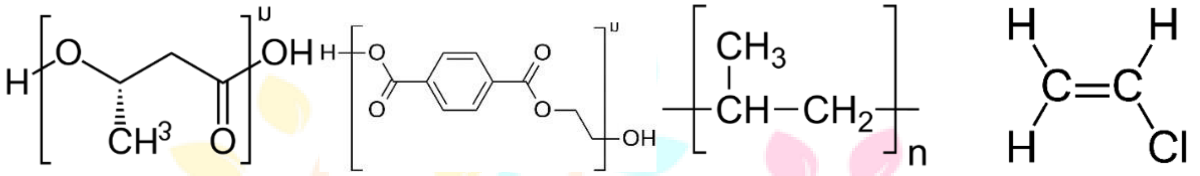
Abstract : Polyhydroxybutyrate (PHB) emerges as a promising biodegradable alternative to petroleum-based plastics, addressing environmental concerns associated with non-degradable materials. This comprehensive review encompasses the structural elucidation and properties of PHB compared to conventional synthetic polymers. It delves into various aspects of PHB synthesis, including fermentation methods and optimization techniques. Additionally, it examines diverse PHB-producing strains and their strain-dependent properties, along with exploring microorganisms capable of PHB synthesis across different taxonomic groups. The influence of carbon sources on PHB production and its thermo-mechanical properties is scrutinized, alongside discussions on downstream processes for PHB recovery. Furthermore, the biodegradation mechanisms of PHB under different environmental conditions are elucidated, emphasizing microbial enzymatic pathways and factors influencing biodegradation rates. Applications of PHB in various sectors, such as food packaging, drug delivery systems, automotive industry, and medical implants, are highlighted for their societal benefits and environmental sustainability. The review concludes with insights into future prospects, emphasizing the need for continued research and innovation to optimize PHB production processes and evaluate sustainability metrics.

Keywords: **- Polyhydroxyalkanoates, biosynthesis, microorganisms, recovery , downstream processes.**

1.INTRODUCTION

Plastic materials derived from petrochemicals pose significant environmental challenges due to their non-degradable nature. Despite being cost-effective, their presence in the environment contributes significantly to environmental degradation. Given the impending fossil fuel crisis and the escalating costs and environmental repercussions of petroleum-based products, there is a critical need to explore alternative solutions to diminish humanity's reliance on finite resources. Biodegradable plastics, such as poly hydroxybutyrate (PHBs), offer a promising solution to mitigate the environmental impact of conventional petroleum-based plastics. PHBs are complex macromolecules synthesized by bacteria, forming inclusion bodies that accumulate as reserve material under different stress conditions [1]. They have properties akin to synthetic thermoplastics like polypropylene and demonstrate versatility suitable for a wide range of applications. However, their elevated production cost is a significant barrier to their widespread production and commercialization. To address this, efforts have been made to reduce the production cost of PHBs through the development of efficient bacterial strains and optimization of fermentation and recovery processes. Enzymes, synthetases, and depolymerases play pivotal roles in the biosynthesis and biodegradation pathways of PHBs and other polyhydroxyalkanoates [2]. These biodegradable polyesters have garnered significant interest due to their potential to serve as substitutes for conventional plastics. This study aims to isolate PHB-producing bacteria and investigate PHB production from waste sources, with the goal of advancing sustainable practices in biodegradable plastic production. Polyhydroxybutyrate (PHB) [3].



[PHB] **[PET]** **[PP]** **[PVC]**

Figure.2 PHB chemical structures in contrast to those of petroleum-based polymers that are often utilized, such as PP, PVC, and PET [4,5].

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 1. An overview of the mechanical characteristics of bio-based polymers (PLA), petrochemical-based polymers (PP, PET, and PE), and P3(HB) is given in the summary [5,13].

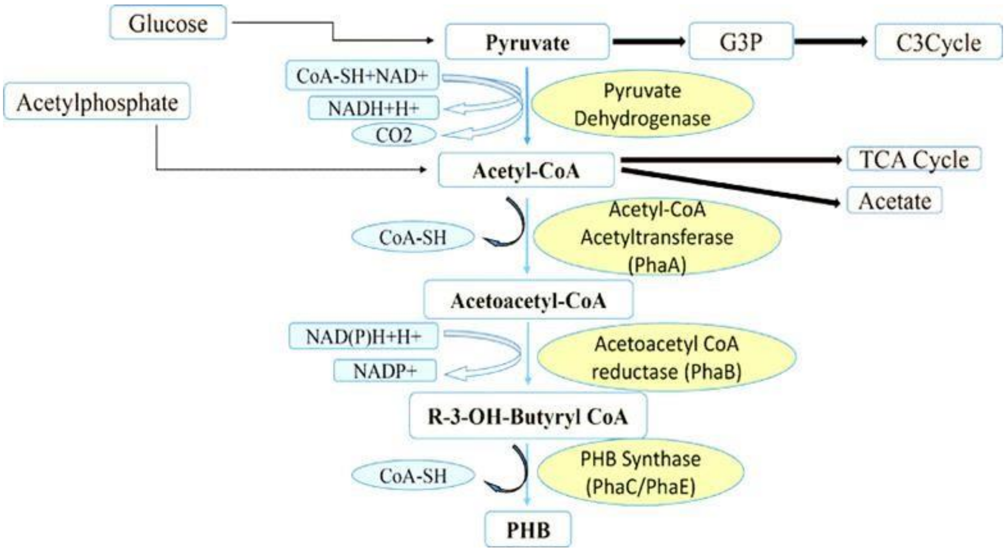


Figure 3 : Biosynthesis pathway of polyhydroxybutyrate (PHB) [6].

Three biosynthetic enzymes assist in the conversion of acetyl-coenzyme-A (acetyl-CoA), which is produced metabolically by bacteria, into PHB.

Step 1: Two molecules of acetyl-CoA are combined to form acetoacetyl-CoA by 3-ketothiolase, a PhaA gene product.

Step II: Acetoacetyl-CoA is reduced by NADH to 3-hydroxybutyryl-CoA by the enzyme acetoacetyl-CoA reductase, which is a PhaB gene product.

Step III: 3-hydroxybutyryl-CoA is polymerized by PHB synthase, a PhaC gene product, to create PHB.

Coenzyme A is released. The only substrates employed by the enzyme responsible for polymerization are (R)-isomers. In healthy development, 3-ketothiolase's ability to exit the TCA cycle is inhibited by free COA.

However, excess acetyl-CoA is directed toward PHB biosynthesis when its input into the Krebs cycle is restricted (during non-carbon dietary constraint) [6].

2. PHB Fermentation Methods:

PHBs can be produced using a variety of fermentation techniques, such as continuous procedures like continuous fed-batch [7] systems employing gaseous substrates or discontinuous techniques like batch culture, fed-batch culture, and repeated fed-batch culture [8]. Continuous processes, however, differ fundamentally from intracellular products such as PHB. Because of the low concentration of carbon and nitrogen inputs, batch culture is one of the least productive simple discontinuous processes utilized to produce PHB. It has been determined that a straightforward "repeated batch" strategy can increase volumetric productivity. The drain-and-fill method of repeated-batch culture was used in study, which effectively raised volumetric productivity and demonstrated a significant advantage over straightforward batch methods in PHB production. Additionally, by using this method, ineffective time is saved on maintaining, replenishing, and sanitizing the bioreactor in between individual batches [9].

When the concentration drops below a certain point, feed-batch systems add precursor substrate using pulses. However, if an appropriate feeding plan isn't followed, this technique might not be more productive than batch culture methods [10]. The material being processed, like PHA, determines the best feeding rates for carbon and nitrogen sources. For a large proportion of accumulated PHB to be successfully produced, substrate concentration control is essential. Several feeding tactics, such as a two-feeding-pulse fed-batch strategy and continuously feeding approaches, have been reported to improve the fed-batch approach [11,12]. Because the bioreactor must be prepared and treated, discontinuous procedures are less productive because the quality of the final output varies from batch to batch. When it comes to producing PHB, continuous feeding is seen to be the most straightforward and optimal approach as compare to other methods [13].

Continuous fermentation systems maintain constants in pH, nutrient supply, and product concentration by operating under stable, controlled circumstances. These techniques are applied in sectors like as the beverage industry, where Lactobacillus amylovorus may convert starch to lactic acid [14]. In contrast, a sufficient number of active cells must grow in the first stage of polyhydroxybutyrate (PHB) under nutritionally balanced conditions, and in the second stage, they accumulate the biopolymer as an intracellular product [15]. As a result of microorganisms' physiological stress reaction to scarce or depleted critical nutrients, only a tiny portion of accumulated material is produced [16]. Consequently, continuous two- and multi-stage fermentation procedures are more productive and offer steady processing conditions when manufacturing PHA materials [17].

PHB materials can be made more productive through continuous fermentation methods, but industry adoption of these techniques has been hindered by the risk of microbial contamination and financial losses [18]. For smaller facilities, continuous production, however, offers cheaper investment costs and higher volumetric productivity. Moreover, it results in more uniformity and consistency in the quality of the output [19]. Continuous cultivation is more effective for PHB biosynthesis, which improves the performance characteristics of PHB-based bioplastics and increases the efficiency of downstream processing. The selection of microorganisms, the medium used for growth, the availability of carbon, and process variables all affect the quantity and quality of PHB [20].

3. PHB Producing Strains:

Over 300 different bacterial strains can produce PHB materials, with Ralstonia eutropha being the most well-studied [21]. Imperial Chemical Industries initially used this strain to create PHB polymers, which were sold under the Biopol brand [22,23]. However, only a few strains have been effectively employed for commercial synthesis [24]. The molecular weight of the material determines its mechanical and thermal properties, except for values exceeding 1400 kDa, where molecular entanglements and insufficient polymer processing conditions cause a decrease in Tm and degree of crystallinity. PHBs' chemical structure and physical characteristics are also straindependent. Crystallinity is a crucial factor in identifying variations in PHB generated by different strains. Research by found that PHB produced by C. nector and Bacillus megaterium had crystallinity degrees of 44% and 23%, respectively. Understanding the degree of crystallinity (Xc) is essential for determining variances in mechanical properties and the quality dependency on the bacterial strain used [25].

Mechanical Property	**Literature Values**	**PHB from *Bacillus megaterium***	**PHB from *C.nector***
Xc (%)	53.4	23–37	46–53
Tm (°C)	169	151–176	169–175
Tg (°C)	1.1	−1–4	−0.2–0.6

Table 2 . The document presents the mechanical characterisation data of PHBs derived from the culture of two distinct strains [24].

4. Microorganisms used for PHB production:

Microorganisms from diverse taxonomic groups, including eubacteria, cyanobacteria, archaebacteria, and eukaryotes, exhibit the capacity to synthesize bioplastics, notably polyhydroxyalkanoates (PHAs), contingent upon specific nutritional and environmental conditions. Additionally, the emergence of transgenic plants as sources of bioplastics adds a significant dimension to this field. This section provides an overview of microorganisms proficient in producing PHBs.

4.1 Eubacteria:

polyhydroxybutyrate (PHB) accumulation in Bacillus megaterium led to the identification of various bacterial strains associated with PHB synthesis [26,27]. PHB-synthesizing bacteria are mainly from pseudomonas, coryneform, and bacillus groups [28,29]. Bacteria used for PHA production can be categorized into two groups based on culture conditions: those requiring surplus carbon sources and limited nutrient availability, and those capable of accumulating PHA without nutrient limitations [30,31 and 32]. Cultivation conditions significantly influence PHA biosynthesis, and recombinant microorganisms like Escherichia coli have demonstrated remarkable PHA accumulation without nutrient limitations [33,34].

4.2 Cyanobacteria:

Cyanobacteria exhibit the ability to accumulate polyhydroxyalkanoates (PHA) by harnessing CO2 and sunlight as carbon and energy sources. These oxygen-evolving photosynthetic organisms naturally possess PHA synthase enzymes [35]. However, to date, primarily polyhydroxybutyrate (PHB) homopolymer has been identified in most cyanobacteria [36]. Noteworthy examples include Spirulina platensis UMACC 161 and Synechocystis sp. PCC6803, which can accumulate PHB up to 10% of the dry cell weight [37]. Additionally, Synechococcus sp. MA19 and Nostoc muscorum have been observed to produce PHB under phosphate-limited conditions [38]. Studies indicate that Nostoc muscorum exhibits a five-fold increase in PHB production under mixotrophic, chemoheterotrophic conditions with nitrogen limitation compared to photoautotrophic conditions [39]. Leveraging cyanobacteria's ability to produce PHB using solar energy can potentially reduce costs and CO2 emissions.

4.3 Archaea:

Haloarchaeal strains, thriving in high salt concentrations, offer cultivation advantages due to reduced requirements for strict sterility. Their cell walls, stabilized by salt, prevent lysis, rendering recovery of PHB and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) from extreme halophiles more convenient and economical [40,41]. Various genera within Haloarchaea, such as Haloferax, Haloarcula, Haloquadratum, Halorubrum, Halobiforma, Halorhabdus, Halalkalicoccus, Halobacterium, Natrianema, Halostagnicola, Natrinema, Natronobacterium, Natronorubrum, Haloterrigena, Halopiger, and Halococcus, have been documented to produce diverse types of PHAs [42].

5. Effect of Carbon Source Present in Media:

Poly hydroxybutyrate (PHB) production is a cost-effective alternative to petrochemical-derived plastics, but its widespread adoption is hindered by the high cost [43]. To overcome this, researchers are developing efficient bacterial strains, optimizing cultivation parameters, and refining recovery processes [44,45]. A significant portion of production costs, up to 50%, is attributed to the carbon substrates used [46]. Studies have explored alternative, cost-effective substrates, such as vinasse and sugarcane molasses, and waste materials [47]. Recent research has also explored the influence of carbon sources on PHB thermo-mechanical properties, with studies comparing PHB produced from rice bran and glucose-based sources [48]. Organic carbon sources also have been studied, with autotrophic production resulting in distinct thermomechanical characteristics compared to heterotrophic growth [49,50]. Vegetable oils, including waste frying oil, have emerged as potential substrates for PHA production due to their availability and cost-effectiveness [51,52].

6. Effect of Downstream Process:

Polyhydroxybutyrate (PHB) recovery from bacterial cells post-fermentation has been extensively studied for its efficiency and purity [53]. The primary extraction methods use solvents, which yield high-purity materials suitable for medical applications. However, the reliance on solvents increases processing costs, making it a concern for industrial-scale production. Studies have examined the impact of different solvents on the efficiency and purity of PHB recovery, as well as the thermal and mechanical properties of PHB materials found .The PHB materials extracted using ethylene carbonate and DMSO solvents exhibited lower enthalpy of fusion, indicating a more elastomeric or rubbery nature. However, temperature during material recovery significantly impacts PHB properties, particularly thermal properties and molecular weight [54]. Optimal conditions for PHB molecular weight were observed at specific temperatures and heating durations, with deviations resulting in alterations to molecular weight and polymer degradation leading to molecular weight reduction. The polydispersity index, a crucial parameter during downstream processing, dictates the suitability of the final product for specific applications [55,56].

7. Biodegradation of PHB:

The biodegradation capacity of polyhydroxybutyrate (PHB) is a notable feature, rendering it environmentally benign across various settings. As a natural polymer, PHB undergoes complete decomposition into water and carbon dioxide, facilitated by microorganisms prevalent in diverse environments such as soil, water bodies, and sewage systems. Microbial communities, including bacteria and fungi present in soil, sludge, and seawater, produce extracellular enzymes specialized in PHA degradation. These enzymes hydrolyze solid PHB into water-soluble oligomers and monomers, subsequently utilizing them as nutrients [57,58,59].

PHB degradation occurs under both anaerobic and aerobic conditions. Anaerobic degradation yields carbon dioxide, water, and methane, while aerobic degradation produces carbon dioxide and water. Extracellular biodegradation, mediated by extracellular depolymerases, occurs in the surrounding environment. Conversely, intracellular biodegradation, mediated by intracellular depolymerase enzymes within microbial cytoplasm, is another mode of degradation.

Several factors influence the biodegradation process, including microbial activity, polymer composition, molecular weight, crystallinity, temperature, moisture, pH, nutrient availability, and oxygen levels [60,61 and 62]. Most PHB-degrading organisms identified to date are mesophiles, although a limited number can function at elevated temperatures. Actinomycetes exhibit higher PHB-degrading activity compared to thermotolerant and thermophilic Streptomyces strains [63].

Intriguingly, intracellular PHB degradation within living organisms yields nontoxic metabolites such as 3-hydroxybutyrate, naturally occurring in blood, rendering them suitable for medical implant devices. Studies indicate that the addition of plasticizers or polymers can accelerate PHB degradation rates. Conversely, hydrophilic additives enhance hydrolysis by promoting water adsorption [64].

Understanding the mechanisms of biodegradation is crucial in determining the fate of biopolymers in natural environments. Various methods can promote biodegradation, emphasizing its importance in sustainable waste management practices.

Biodegradation, also known as biotic deterioration, is a process where microorganisms break down polymers into more basic molecules. Abiotic degradation techniques like mechanical, oxidative, or hydrolytic decomposition can speed up the process, but they don't discriminate. The rate of biodegradation depends on factors such as hydrophilicity, reduction potential, polymer composition, and chemical structure [64].

Biodegradability is the most promising aspect of PHB, which attracts economic interest. Most soil bacteria can break down PHB in any condition and environment. Microorganisms capable of breaking down PHB can produce the unique enzyme PHB depolymerase, which can be used both inside and outside the cell. Over 600 distinct depolymerase types have been identified to date [64].

Various processes, including biodeterioration, biofragmentation, assimilation, mineralization, and enzymatic degradation by extracellular and intracellular depolymerases, are involved in the degradation of polymers. PHB dimer and 3HB monomer are produced when PHB depolymerase breaks the ester linkages in PHB. Strong depolymerase producers can be extracted from plasticcontaminated areas[66].

PHB is converted into 3-hydroxybutyric acid by enzymes like oligomer hydrolase and PHB depolymerase, which are further oxidized to acetyl acetate by a dehydrogenase enzyme. Pseudomonas, Actinomadura, Penicillium, Aspergillus species, Microbispora, Saccharomonospora, Streptomyces, Thermoactinomyces, and Bacillus species can break down PHAs with or without oxygen. PHAs can be broken down by anaerobic bacteria found in sludge in various settings, but soil is the most natural setting for PHA breakdown [64].

7.1 Anaerobic digestion of PHB:

Anaerobic digestion is a crucial step in wastewater treatment, reducing organic matter in effluent. It can be performed at a regular or higher temperature, and can also be used for methane preparation. This method is effective in eliminating polymers and can fully biodegrade PHA in 20 days under mesophilic conditions. Enzyme activity and temperature have a relationship in anaerobic digestion, with optimal activity at 30°C and no activity beyond 70°C. PHB powder deteriorates by 90% at 37°C in 10 days, according to ISO 13975[65].

8. Benefits of PHB to Society:

Polyhydroxy butyrate (PHB) is a naturally occurring polyhydroxyalkanoate (PHA) and the most common PHA. It is biodegradable in all natural and marine environments. Its annual production capacity is around 30,000 tons, significantly less than global Polypropylene production of 65 million tons. PHB was first introduced in 1982 [67].

9. Applications of PHB

9.1 Food packaging:

When the PHB was successfully evaluated for use in food packaging, it was shown to be less flexible and stiffer than polypropylene. While researching the impact of pasteurization on a pork salad packaged in a PLA or PHB bio-based container and a polyethylene or polypropylene container was examined. PHB films, the researchers discovered, can be utilized to package this kind of food. It has been shown that PHB may completely replace polypropylene in the packaging of fatty goods, such as cream cheese, margarine, and mayonnaise. Additionally, the researchers examined a variety of factors, including dimensional, mechanical, sensory, and physical data. PHB's nanoparticles contribute to the enhanced food packaging's qualities [67,68].

9.2 Drug delivery system:

PHB's natural materials qualities, such as their biodegradability and thermostability, make them a desirable option for application in tissue engineering, drug administration, and traditional medical devices. Biodegradable polymers can be used to encase drugs, which can then be absorbed by the body. This might be an effort at continuous medication release over several months for localized drug delivery. PHB is ideal for this event because of its capacity to break down in the tissues of the host organism, releasing bioactive molecules that may be an antibiotic or an anticancer medication. PHB may also be made into films, porous matrices, microcapsules, microspheres, and nanoparticles since it is hydrophobic and biocompatible. It can therefore function as a productive medication delivery device [57].

9.3 Automotive industry:

The bio-based materials are attracting interest from the automotive industry. It has emerged as a novel engine concept using bio-based parts. Here, it is important to remember that industries are becoming more and more in need of biofuel. PHB may be treated to create biodegradable materials including film, fiber, and scratch-resistant surfaces [69].

9.4 Implant:

Just eight PHAs, including PHB, are generated in sufficient quantities to be studied as medical implant materials out of all those that are now accessible. It ages quickly and has a robust structure. The FDA has authorized the use of PHB in suture making. PHB satisfies the criteria for an appropriate medical device, which is long-term interaction with human body tissues without causing injury. Numerous adverse consequences and a second procedure are necessary for the majority of permanent implants. Regarding PHB, none of these things are real. PHB was successfully used as a temporary scaffold for the regeneration of pericardial tissues in sheep used in a trial where it was placed as a pericardial patch. PHB has applications in bone engineering as well [69].

10 Challenges:

Achieving cost competitiveness with conventional petrochemical-based plastics is a difficulty for PHB manufacture. To make PHB commercially feasible on a wide scale, downstream processing and substrate cost optimization are required. Improving yield per unit of substrate is critical, because high yields and productivity depend on maintaining ideal fermentation conditions. Utilizing waste materials and inexpensive, renewable feedstocks can improve sustainability and financial viability. PHB yields and quality can be decreased by undesirable bacteria contaminating the bioprocess. For output to be constant and dependable, strong fermentation systems with efficient contamination control mechanisms must be developed. The commercialization of PHB is influenced by market demand and regulatory frameworks, which provide considerable hurdles [69].

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