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Poly(3-hydroxybutyrate) Production from Wastewater: Microbial Pathways, Production Strategies, and Sustainability Perspectives

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ABSTRACT: The increasing accumulation of petroleum-based plastic waste and wastewater has intensified the need for sustainable waste management and biodegradable alternatives. Poly(3-hydroxybutyrate) (P3HB), a microbial biopolymer, has emerged as a promising substitute for conventional plastics. This article reviews the potential of wastewater as a renewable substrate for P3HB production, with emphasis on microbial pathways, production strategies, and sustainability aspects. It discusses different wastewater sources, P3HB-producing microorganisms, metabolic engineering approaches, production processes, and polymer recovery techniques, highlighting the factors that influence productivity and product quality. The integration of P3HB production into biorefinery systems and its contribution to resource recovery, greenhouse gas mitigation, and the circular bioeconomy are also addressed. Overall, wastewater-based P3HB production represents a sustainable and economically attractive approach for biodegradable polymer production, although further technological advances are required to support large-scale industrial implementation.

KEYWORDS: Biopolymer production, Circular bioeconomy, Poly(3-hydroxybutyrate) (PHB), Wastewater valorization, Microbial pathways, Sustainability.

1. INTRODUCTION

Global warming, greenhouse gas (GHG) emissions, air and water pollution, climate change, and their associated health impacts have become critical challenges for researchers worldwide. The persistent reliance on fossil fuel–derived materials is a major contributor to rising GHG emissions and the accelerated consumption of natural resources. Plastics, primarily produced from non-renewable feedstocks, pose significant environmental and health risks throughout their life cycle. In 1950, global plastic production was estimated at 1.5 million metric tons (Mt), and it has grown rapidly due to extensive use in both daily life and industrial applications. By 2020, global plastic output exceeded 367 million Mt, with projections suggesting it could reach 34 billion Mt by 2050—a dramatic increase reflecting a more than twofold growth between 1989 and 2002.(Al et al., 2024). The escalation of plastic production has markedly exacerbated plastic pollution in both terrestrial and marine environments. In 2010, approximately 275 million metric tons of land-based plastic waste were generated by 192 coastal countries, with an estimated 4.8 to 12.7 million metric tons entering the oceans. Without substantial enhancements in waste management infrastructure, these amounts are projected to rise sharply by 2025, thereby intensifying the ongoing environmental crisis.(Lai et al., 2022). Furthermore, as of 2015, only 9% of the 6.3 billion metric tons of plastic waste generated had been recycled, while 12% was incinerated; the remaining majority accumulated in landfills or leaked into natural environments. If current production and waste management trends persist, plastic waste is projected to reach 12 billion metric tons by 2050(Ji et al., 2023). Moreover, greenhouse gas (GHG) emissions associated with plastics extend beyond their production and processing, occurring throughout the entire life cycle, including manufacturing, refining, waste management, and disposal. By 2050, plastics are projected to account for approximately 13% of the global carbon budget allocated for climate change mitigation. Plastics are highly persistent in the environment; for example, a typical plastic bottle may require around 450 years to degrade completely, plastic bags approximately 25 years, and fishing nets up to 600 years. Consequently, plastic waste constitutes one of the most pressing environmental challenges of our time. Throughout its life cycle, it imposes a substantial toxic chemical burden, underscoring the urgent need to reduce plastic production at the source. Achieving carbon neutrality by 2050 and implementing effective environmental policies necessitate coordinated efforts from multiple stakeholders, including industries, researchers, non-governmental organizations (NGOs), policymakers, and the general public. To address plastic pollution and

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enhance understanding of the socio-economic dimensions of environmental protection, the European Union has initiated comprehensive measures such as the Green Deal and Agenda 2030.(Lai et al., 2022).

In recent years, bioplastics have attracted significant attention as sustainable alternatives capable of replacing conventional polymers in various applications. They are generally classified as bio-based, biodegradable, or both (Koch, Berendzen, et al., 2020). Bioplastics, particularly for packaging, provide environmentally friendly solutions with properties comparable to traditional plastics. A notable category is microbial bioplastics, consisting of water-insoluble polymers synthesized within the cytoplasm of certain microorganisms. These biodegradable intracellular inclusions function as carbon and energy reserves and protect cells against osmotic stress(Koch, Bruckmoser, et al., 2020). Among microbial biopolymers, poly(3-hydroxybutyrate) (P3HB) are accumulated as intracellular granules in bacterial cells, serving as energy and reducing power reservoirs. The most studied (P3HB) is polyhydroxybutyrate (PHB), an aliphatic polyester synthesized under nutrient-limiting conditions and stored in cytoplasmic granules(R et al., 2021). (P3HB) production occurs primarily in bacteria, although diatoms and green algae can also synthesize it. Genetically engineered bacteria have shown enhanced polymer yields, and research has focused on metabolic pathways and genetic modifications to optimize production, which typically proceeds when carbon is abundant but nutrients like nitrogen or phosphate are limited (Lai et al., 2022).

Poly(3-hydroxybutyrate) ((P3HB)s) are inherently hydrophobic, biocompatible, and exhibit high oxygen impermeability, stability under extreme conditions, and UV resistance(Ilhami et al., 2025). These properties make (P3HB) suitable as biopolymers, particularly for food packaging. However, high production costs limit their global application (Jeyaraj & Vishnu Priya, 2022). Factors such as carbon source cost, downstream processing, and production methods contribute significantly to overall expenses, with biopolymers costing $6–$15/kg compared to $0.23–$0.48/kg for conventional plastics like polyethylene (PE) and polypropylene (PP) (Koch, Berendzen, et al., 2020).Raw materials, especially carbon sources for microbial growth, account for approximately 70–80% of production costs.

Urbanization and industrialization have increased wastewater (WW) generation, which, if discharged untreated, poses environmental risks such as eutrophication(Kumari et al., 2024). Nevertheless, certain wastewaters serve as nutrient-rich substrates for microbes, including bacteria, cyanobacteria, and fast-growing eukaryotic algae (Mastropetros et al., 2022). Microbial wastewater treatment (WWT), as in activated sludge systems, not only mitigates pollution but also supports microbial growth and resource recovery (Agarwal et al., 2022 & Ge & Champagne, 2016)These nutrients can facilitate the production of biomethane, biofuels, natural antioxidants, biofertilizers, and biopolymers (Das et al., 2018).

(P3HB) production remains 4–9 times more expensive than polyethylene due to requirements for pure culture fermentation, specific substrates, and controlled culture conditions(Rueda et al., 2024).Optimization of parameters such as temperature, pH, light, nutrients, and cycle duration is critical for maximizing yields (Ansari & Fatma, 2016). Traditional (P3HB) carbon sources are carbohydrates (sucrose, maltose, glucose, starch), fatty acids, methanol, and alkanesIntegrating WWT with (P3HB) production has emerged as a promising strategy to utilize cost-effective, non-competitive carbon sources, reducing both production costs and environmental impact (Khatami et al., 2021 &Amadu et al., 2021). By combining microbial WWT with resource recovery, the environmental footprint of wastewater can be minimized, and the cost of producing valuable microbial bio-products, such as bioplastics, can be significantly reduced.

2. MICROBIAL MECHANISMS OF (P3HB) BIOSYNTHESIS

2.1. Optimization of Microbial Strains

Microorganisms capable of producing poly(3-hydroxybutyrate) (P3HB) demonstrate wide ecological and physiological diversity, thriving in environments such as dairy waste, oil-processing residues, agricultural by-products, pulp and paper effluents, and wastewater sludge (Jeong et al., 2021). Among them, halophiles have attracted attention for their resilience in non-aseptic and extreme conditions. Identification of (P3HB)-producing bacteria from natural habitats typically involves sampling followed by phenotypic and genotypic screening (Al et al., 2024). Microbial producers are generally classified by ecological niche, cell wall composition, and culture requirements. One group, including Pseudomonas oleovorans (Brigham et al., 2010) synthesizes (P3HB)s under excess carbon and limited essential nutrients (e.g., nitrogen, phosphorus, sulfur, magnesium). Another group, such as recombinant Escherichia coli and Alcaligenes latus, accumulates (P3HB) even during active growth without nutrient limitation. Most reported strains are Gram-negative, while Gram-positive producers remain less common (Mastropetros et al., 2022). A notable

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example is Ralstonia eutropha (formerly Alcaligenes eutrophus), a soil-inhabiting Gram-negative bacterium of the β-Proteobacteria. First isolated in Germany, this strain can accumulate (P3HB)s up to 90% (w/v) of its dry cell weight, alongside related compounds like hydroxyalkanoic and mercaptoalkanoic acids (Müller-Santos et al., 2020).

Owing to its broad substrate spectrum and capacity for high polyhydroxyalkanoate (P3HB) accumulation, Ralstonia eutropha has become one of the most extensively studied (P3HB)-producing bacteria(de Mello et al., 2023) This versatile microorganism can metabolize diverse carbon sources, including formic acid, fructose, and CO_2, and supports (P3HB) biosynthesis under both heterotrophic and autotrophic cultivation. During heterotrophy, organic substrates are consumed with CO_2 released as a byproduct. Nutrient conditions strongly regulate (P3HB) synthesis: while excess ammonium suppresses accumulation, nutrient limitation redirects metabolic flux toward polymer biosynthesis (Janasch et al., 2022). Cyanobacteria also represent promising (P3HB) producers due to their minimal nutrient requirements and ability to accumulate (P3HB) under photoautotrophic conditions. Harnessing solar energy, they convert waste CO_2 into biodegradable polymers (Sharma et al., 2016). Genera such as Aphanothece, Gloeothece, Spirulina, and Synechococcus have demonstrated this potential. These organisms thrive in diverse environments and assimilate inorganic nitrogen and phosphorus from sources such as industrial effluents, aquaculture wastewater, sewage, and agricultural runoff (Ajala & Ojoawo, 2022) Although smaller cell size can limit biomass yields, their (P3HB)-synthesizing capacities remain comparable to other microbial candidates.

2.2. Effect of Stress Conditions on (P3HB) Production

Nutrient availability, especially carbon and nitrogen sources, plays a central role in both the accumulation and composition of poly(3-hydroxybutyrate) (P3HB). Carbon sources, typically sugars, enhance (P3HB) synthesis, while nitrogen sources (e.g., ammonium salts, organic compounds) regulate growth and determine the biomass-to-(P3HB) ratio. Optimal yields depend on maintaining a balanced C/N ratio, with additional nutrients such as sulfur, phosphorus, and trace elements further influencing biosynthesis (de Mello et al., 2023). Under nitrogen or phosphorus limitation, bacteria undergo metabolic reprogramming that redirects surplus carbon into intracellular (P3HB) granules, serving as carbon and energy reserves. These stores can later be mobilized to support growth when nutrient conditions improve (Das et al., 2018).

Different nitrogen forms exert distinct influences on microbial growth and polyhydroxyalkanoate ((P3HB)) synthesis. Nitrate has been shown to be a more effective limiting nutrient than ammonia for enhancing yields, while organic sources such as peptone often promote higher production compared to inorganic compounds like ammonium chloride (Rueda et al., 2024 & Kumari et al., 2024).). Optimizing the carbon-to-nitrogen (C/N) ratio near nutrient-limiting thresholds further improves accumulation. In microalgae, nitrogen limitation reduces growth and alters biochemical composition; however, supplementation with additional carbon sources can mitigate these effects. Glucose enhances both biomass and (P3HB) production, whereas acetate specifically boosts intracellular accumulation under nitrogen-deficient conditions, particularly when added early in cultivation (Jeong et al., 2021). Microbial (P3HB) production depends strongly on the availability of suitable substrates and metabolic precursors. Common carbon sources—sucrose, fructose, glucose, acetate, and propionate—are metabolized to acetyl-CoA, the central intermediate in (P3HB) biosynthesis. The type and concentration of these substrates critically determine productivity, making their optimization essential for maximizing microbial yields(Wang et al., 2025).

2.3. The Effect of Micronutrients on (P3HB) Biosynthesis

The (P3HB) production is heavily dependent on micronutrients. (P3HB)-producing bacteria require sufficient micronutrients for their growth and metabolic processes. Essential micronutrients, such as iron, zinc, copper, and manganese, play a critical role in enabling these microorganisms to produce PHB and carry out various other metabolic functions(Wang et al., 2025). A deficiency in vitamins can hinder microbial growth and (P3HB) synthesis, resulting in lower yields and diminished polymer quality. In A. australica, it has been shown that phosphates, $MgSO_4 \cdot 7H_2O$, and trace elements (iron, calcium, cobalt, zinc, manganese, nickel, and copper) significantly influenced both biomass and PHB production. Maintaining the nutritional medium's buffering capacity, which is essential for microbial growth and (P3HB) generation, requires minerals. Enzyme activity and the preservation of protein structure depend on trace elements. Since PHB production occurs during the development phase of A. australica culture, it is important to optimize the concentration of these components (R et al., 2021).

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2.4. Factors Affecting ((P3HB)) Accumulation

According to multiple studies, the overall cost of biopolymer production is determined not only by substrate expenses but also by microbial productivity, cultivation conditions (e.g., pH, aeration, temperature), and the efficiency of downstream recovery and purification processes (Dietrich et al., 2020). The type of substrate and the method of its application substantially influence microbial community performance, while cellular physiological activities are tightly regulated by environmental factors such as light, temperature, and nutrient availability. In closed cultivation systems, these parameters can be precisely controlled, thereby ensuring optimal microbial growth and (P3HB) accumulation. By contrast, open pond systems face significant challenges in maintaining such control, primarily due to fluctuations in external environmental conditions. The ability of (P3HB)-producing microorganisms to accumulate large amounts of polymer, as well as the composition of the synthesized material, is strongly affected by nutrient availability, pH, temperature, feeding strategies, cycle duration, and exposure to light. Optimization of culture parameters—including temperature, pH, light intensity, nutrient concentrations, and cultivation duration—is therefore critical for achieving maximum yields. Moreover, the intrinsic properties of microbial strains, including their metabolic potential, nutrient uptake efficiency, and strain-specific characteristics, must be considered when predicting (P3HB) production. Notably, the molecular weight of PHAs synthesized within bacterial cells is highly sensitive to surrounding cultivation conditions, underscoring the importance of fine-tuned process control (Kumari et al., 2024).

3. (P3HB) PRODUCTION FROM WASTEWATER-DERIVED SUBSTRATES

Since the 1950s, microbial cultivation using wastewater (WW) has been employed to achieve dual objectives: wastewater treatment and the production of valuable microalgal biomass, thereby promoting resource recovery. Wastewater has long served as a substrate for synthesizing various bioproducts, including biofuels, lipids, and biopolymers (Amadu et al., 2021). At the laboratory scale, poly(3-hydroxybutyrate) (P3HB) have been successfully produced from diverse waste streams. While substrate cost is a major factor in the overall economics of biopolymer production, microbial productivity, culture conditions (e.g., temperature, aeration, pH), and recovery and purification strategies also critically impact total production costs. Carbon sources alone are estimated to account for 25–45% of production expenses. Accordingly, low-cost substrates such as methanol, cheese whey, molasses, olive oil mill waste, and poultry waste have been applied in commercial (P3HB) production. These feedstocks are generally categorized as industrial or municipal wastewater. Two primary strategies exist for converting wastewater into (P3HB)s: (i) using wastewater as a carbon source for cultivating a pure culture of a specific microorganism, and (ii) employing an open mixed microbial culture (MMC) selectively enriched with (P3HB)-producing bacteria (Mastropetros et al., 2022). The latter approach adapts conventional biological wastewater treatment methods, such as activated sludge, to enable simultaneous (P3HB) accumulation and wastewater remediation. Furthermore, anaerobic digestion and other sustainable waste management techniques generate digestate supernatant and effluents that serve as carbon, nitrogen, and phosphorus sources for microalgal cultivation. Key quality improvement indicators, particularly reductions in nitrogen, phosphorus, and chemical oxygen demand (COD), are summarized in Table 1.

3.1. Biotechnological Production of (P3HB) Using Industrial Wastewater

Food processing wastewater offers a cost-effective substrate for microalgal cultivation due to its high content of fermentable nutrients, including lactose, lipids, and soluble proteins (Samantaray & Nayak, 2011). The utilization of complex substrates can be further enhanced by employing mixed microbial cultures. Phosphorus-accumulating organisms (PAOs), widely used in wastewater treatment (WWT) for phosphorus removal, can synthesize poly(3-hydroxybutyrate) ((P3HB)s) as intracellular energy reserves (Kamravamanesh et al., 2017). The overall cost of (P3HB) production can be significantly reduced through the use of carbon-rich organic wastes from industrial sources, such as municipal, brewery, and agricultural effluents. Integration of biopolymer production into wastewater treatment has been demonstrated in the sugar industry using two simultaneous sequencing batch reactors (SBRs), which efficiently generated biomass and (P3HB) while maintaining effluent standards for carbon, nitrogen, and phosphorus. Chemoautotrophic bacteria, such as Cupriavidus sp. KKU38, have shown the ability to utilize cassava starch wastewater (CSW) as a substrate for (P3HB) production(Amadu et al., 2021) Acidogenic fermentation of CSW to produce volatile fatty acids (VFAs) provides a more efficient route for (P3HB) synthesis compared to untreated CSW. Studies investigating variations in the chemical oxygen demand to nitrogen to phosphorus (COD:N:P) ratio identified the optimal ratio of 100:0.5:11, resulting in the highest (P3HB) yield of 85.53%. While wastewater demonstrates substantial potential as a substrate for (P3HB) production, achieving optimal yields clearly depends on careful optimization of culture conditions (Kavitha et al., 2016).

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3.2. (P3HB) Biosynthesis Utilizing Municipal Wastewater

The concentration and composition of wastewater (WW) components critically influence polyhydroxyalkanoate (P3HB) biosynthesis. For instance, when glucose is used as the sole carbon source, it is mainly metabolized via the succinate–propionate pathway, producing propionyl-CoA and poly(3-hydroxyvalerate) (PHV) rather than (P3HB). Combining carbon-rich industrial waste with municipal wastewater (MW) has been proposed to provide a more efficient substrate, enhancing growth, nutrient assimilation, photosynthetic efficiency, and (P3HB) accumulation, as demonstrated in Chlorella sorokiniana, with an estimated economic return of $75.6 per kilogram of biomass (Mastropetros et al., 2022). Conversely, protein-rich substrates such as beef extract resulted in low (P3HB) accumulation (~13% CDW), indicating inefficiency of polyphosphate-accumulating organisms (PAOs) in metabolizing amino acid-rich feedstocks. Innovative wastewater treatment strategies, such as combining nitritation/denitritation with aerobic enrichment of (P3HB)-accumulating microbial communities, allow microorganisms to store (P3HB) efficiently while supporting denitrification during nutrient starvation phases. A major challenge in using mixed microbial cultures (MMC) is variability in carbon source preference among species, which can reduce overall (P3HB) yield due to non-contributing organisms. Enrichment strategies under nutrient-limiting conditions selectively favor high-yielding (P3HB) producers, sometimes resulting in nearly pure cultures. For example, repeated cycles of carbon and nitrogen limitation have led to the dominance of Pseudomonas species. Enriched MMCs have shown superior (P3HB) production from short-chain fatty acids (SCFAs) compared to pure strains, with significantly improved yield per unit of volatile fatty acid (VFA) consumed. However, low VFA concentrations in municipal wastewater (MWW) remain a challenge, which can be mitigated by feast-famine operational strategies at both laboratory and pilot scales (Hammond et al., 2025),). One key advantage of MMCs is their robustness in non-sterile environments, unlike pure cultures that require strict aseptic conditions. Compared to conventional biogas and bioenergy recovery technologies, techno-environmental evaluations suggest that (P3HB) production from MMCs in municipal wastewater treatment plants (WWTPs) offers a promising route for generating higher-value renewable raw materials. Table 1 summarizes key studies on microbial (P3HB) production using wastewater-derived cultures, highlighting the dual benefits of producing economically valuable biopolymers while improving wastewater treatment performance.

3.3. Bioconversion of Wastewater into (P3HB) and Value-Added Bioproducts

In addition to the exclusive production of poly(3-hydroxybutyrate) ((P3HB)s) using wastewater (WW) as a nutrient source, the cell factory approach enables the co-production of additional valuable metabolites. In microbial systems, (P3HB) synthesis often occurs alongside other metabolic products, as the (P3HB) biosynthetic pathway interacts with and influences other metabolic routes (Basset et al., 2016). The dominant product, however, depends on the microbial strain, culture conditions, and specific study objectives. When biosynthetic pathways do not compete for the same substrates, integrated strategies to maximize multiple bioproduct yields are feasible. Growth conditions can be adjusted to favor accumulation of specific metabolites, and for efficient downstream processing—particularly in simultaneous extraction—one product should ideally be membrane-bound, secretory, or extracellular(Bengtsson et al., 2017). (P3HB) can be co-produced with carotenoids such as astaxanthin and β-carotene, which hold high commercial value in food, alternative medicine, and nutritional supplements. A zero-waste production approach for these metabolites offers significant potential to reduce overall costs. For example, Rhodobacter sphaeroides can utilize waste effluents to produce both hydrogen and (P3HB)s while lowering chemical oxygen demand (COD) levels (Valentino et al., 2017). Similarly, Rhodobacter palustris CGA676 co-produces (P3HB) and hydrogen using agro-industrial waste. Maximum (P3HB) yield has been reported with olive pomace (11.53% total solids), while highest hydrogen production was achieved using wheat bran effluents (648.6 mL/L) (Kumari et al., 2024), The co-production of multiple metabolites from a single batch culture demonstrates substantial potential for cost reduction. Over the years, genetic manipulation strategies have been developed to enhance polymer content, reduce production costs, and improve polymer quality, progressively refining microbial systems to meet biotechnological demands.

Table 1 : Biopolymers Production Using Microorganisms Cultivated in Wastewater

Microorganisms	Substrate	(P3HB) content	References
Purple non-sulfur bacteria (mixed consortium)	Winery WW	203 mg/L	(Policastro et al., 2020)
Cupriavidus necator	Cane final molasses	2.86 ± 0.82 g 27%	(Sen et al., 2019)
Chlorella pyrenoidosa	Cheese whey WW	6.54g/L (79.8%)	Sathya et al., 2018)

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Activated sludge	Rice & Jowar grain-based distillery spent wash	40% - 42.3%	(Khardenavis et al., 2007)
Activated sludge	Food processing industrial WW (acetic)	33%	(Kumar et al., 2004)
Azohydromonas lata DSMZ 1123	Dairy industrial WW (Cheese Whey)	P(3HB) 1.21 g/L; P(3HV) 0.45 g/L	(Sharifzadeh et al., 2010)
Activated sludge	Simulated WW(Acetate)	55%	(Ozdemir et al., 2014)
sludge	Cheese WW	3% by MLVSSk	(Goffredo et al., 2009)
MMCh (Activated sludge)	Synthetic WW (acetate, yeast extract)	28.8–50%	(Rodgers and Wu, 2010)
E. coli	Hydrolyzed microalgae Supernatant with standard E. coli M9 growth media	31% PHB	(Rahman et al., 2015)
Bacillus subtilis nG220	Sugar industry WW	51.80%	(Singh et al., 2013)
Synechocystis cf. salina Wislouch (No. 192)	Digestate supernatant	78% PHB	(Meixner et al., 2016)
Aulosira fertilissima	Aquaculture WW	92 g/m2 (summer), 89 g/m2 (rainy), 80 g/m2 (winter)	(Samantaray et al., 2011)
Bacillus megaterium CCM2037	Cheese whey	51.57%	(Obruca et al., 2011)
Pseudomonas aeruginosa	Sugar refinery waste (cane molasses)	62.44% CDWe PHB	(Tripathi et al., 2012)

4. PROCESSES FOR PRODUCING (P3HB)

The first and most critical step in (P3HB) production is the selection of an appropriate microbial strain, specifically one recognized for its high capacity to accumulate (P3HB)s (Fig. 1). Once the strain has been identified, it is cultivated under controlled conditions that promote (P3HB) biosynthesis through the utilization of a carbon-rich substrate. Renewable feedstocks, such as wastewater and industrial by-products, provide cost-effective and sustainable substrates for this process. During fermentation, the selected microorganism metabolizes the available carbon source, resulting in the intracellular accumulation of (P3HB) granules, which serve as reserves of both carbon and energy. Upon completion of fermentation, (P3HB)s are extracted and purified from the microbial biomass, with downstream recovery processes designed to remove impurities and other cellular components to ensure high product purity (Kumari et al., 2024). The purified (P3HB) can then be processed into multiple forms—including granules, films, or composites—that are applied across a wide range of industrial sectors. In particular, (P3HB)s have gained significant attention as eco-friendly alternatives in packaging and related sustainable material solutions. The following sections provide a comprehensive discussion of the (P3HB) production process and the diverse factors that influence overall yield and product quality.

FACTORS AFFECTING (P3HB) SYNTHESIS AND COMPOSITION

The physiological activities of microbial cells are strongly influenced by environmental parameters such as temperature, light intensity, and nutrient availability. While these factors can be precisely regulated in closed cultivation systems, achieving similar control in open pond systems remains a considerable challenge due to external fluctuations (Janasch et al., 2022). Within optimal ranges, however, these parameters support maximum microbial growth and metabolite production. Key culture conditions—including nutrient availability, feeding strategies, pH, cycle duration, temperature, and light exposure—affect not only the composition of poly(3-hydroxybutyrate) (P3HB) but also the ability of (P3HB)-producing microorganisms to accumulate large quantities of the polymer. In evaluating (P3HB) yield, it is essential to account for strain-specific physiological traits, such as nutrient

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uptake efficiency, metabolite production capacity, and unique metabolic behaviors. For example, in Escherichia coli, variations in growth conditions have been shown to significantly alter the molecular weight of the synthesized (P3HB), underscoring the importance of environmental optimization in determining both polymer quality and productivity (Wang et al., 2025).

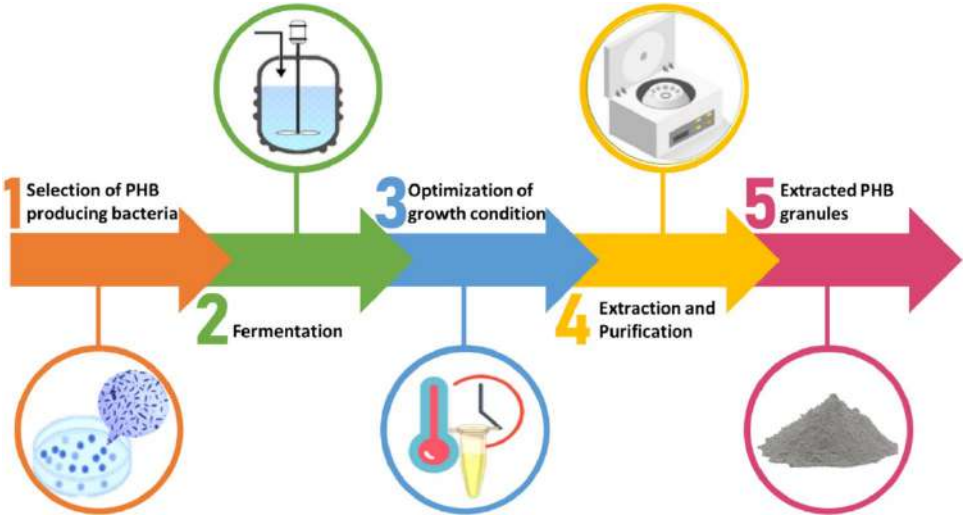

Figure 1: Processes for Producing (P3HB)

4.1. Strain

The type and application method of the substrate, alongside its cost, play a crucial role in determining microbial performance during polyhydroxyalkanoate (P3HB) production, with substrate expenses remaining a major economic barrier (Feng et al., 2023). For instance, cyanobacteria species such as Synechococcus, Leptolyngbya, and Oscillatoria cultivated in ASN III medium demonstrated faster growth than green microalgae (Chlorella), with cyanobacteria peaking on day 18 compared to day 21 for Chlorella. These growth differences highlight the importance of strain specificity under controlled conditions, further supported by variations in the thermal stability of the extracted (P3HB)s. The use of Mixed Microbial Cultures (MMCs) in activated sludge systems has proven advantageous, as it not only reduces production costs but also enhances polymer biodegradability (87%) compared to pure culture systems (Koch, Berendzen, et al., 2020).). To improve biopolymer yield, enrichment strategies are often applied to suppress non-storing populations. For example, the presence of Methylobacillus flagellates reduced the maximum (P3HB) content to 66 wt%, compared to over 80 wt% in Sequencing Batch Reactors (SBRs) (SBR)(Bengtsson et al., 2017). Further studies revealed that in MMCs dominated by Proteobacteria, Plasticicumulans acidivorans, and Thauera selenatis, (P3HB) levels of 84–90 wt% could be achieved (Amadu et al., 2021). Implementation of a feast/famine regime using lactate and acetate demonstrated selective pressure favoring (P3HB)-storing strains. Similarly, investigations using fermented sugar molasses showed that an influent concentration of 45 cmmol VFA/L resulted in an enriched (P3HB)-storing population (88%) and a production yield of approximately 74.6%. Under these conditions, neither the substrate concentration nor the feast-to-famine ratio was a limiting factor, confirming that the dominant microbial community was primarily responsible for the observed (P3HB) accumulation (Feng et al., 2023).

4.2. Nutrients

Nutrient limitation is a critical factor influencing polyhydroxyalkanoate (P3HB) biosynthesis, particularly nitrogen (N) and phosphorus (P) stress, which have been widely studied for their role in enhancing PHB accumulation(Afreen et al., 2021& Markl et al., 2018). The combined shortage of N and P in Synechocystis sp. PCC 6714 under photoautotrophic conditions resulted in maximum PHB storage and favored cyanobacterial dominance over Scenedesmus sp. in mixed consortia sequencing batch reactors (SBRs). Such observations highlight the link between nutrient stress and strain selectivity. Under nutrient deprivation, especially nitrogen, microorganisms undergo metabolic reprogramming, characterized by chlorophyll degradation, reduced protein synthesis, and increased storage polymers such as glycogen and PHB (Lai et al., 2022). When essential nutrients (N, P, Mg, or K) are lacking, cells divert excess carbon toward PHB accumulation, which later serves as a reserve once nutrient balance is restored (Afreen et al., 2021). Notably, nutrient limitation, rather than complete starvation, is generally more favorable for PHB synthesis. The form of

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nitrogen also shapes PHB yields, with nitrate outperforming ammonia and organic nitrogen (e.g., peptone) being more effective than inorganic sources (NH_4Cl) (Feng et al., 2023). Among 63 bacterial strains, nitrogen deprivation induced higher PHB levels compared to phosphate or potassium deprivation. However, nitrogen limitation often reduces algal biomass, prompting supplementation with additional carbon sources. For instance, acetate supplementation in Synechocystis PCC 6803 doubled PHB accumulation, while glucose improved both growth and productivity (Bengtsson et al., 2017).

At the molecular level, nitrogen starvation downregulates genes involved in assimilation, with regulators SigE and Rre37 playing pivotal roles. Similarly, phosphorus limitation in mixed cultures led to cyanobacteria-dominated systems with enhanced carbohydrate content compared to N- or C-limited environments. Optimized nutrient feeding strategies thus emerge as key tools to maximize carbon uptake and PHB productivity in microbial systems (da Silva Moura et al., 2019).

4.3. The Method of Feed (P3HB)

The feeding strategy applied in controlled cultivation systems plays a pivotal role in determining microbial bio-product accumulation. Under mixotrophic conditions, for instance, late-exponential supplementation with glycerol was shown to yield the highest biomass and lipid productivity. In a similar context, (P3HB) biosynthesis is frequently more effective in two-stage cultivation. During this process, cells initially proliferate in a nutrient-rich medium, and once sufficient biomass is achieved, they are transferred into a nitrogen-depleted environment. The lack of nitrogen or phosphorus subsequently induces (P3HB) accumulation, diverting metabolic activity from biomass expansion toward polymer storage (da Silva Moura et al., 2019). In contrast, one-step cultures relying solely on glucose are constrained by nitrogen availability, which restricts cellular growth while channeling excess carbon into (P3HB) synthesis. Aerobic dynamic feeding (ADF) further offers a promising strategy to enhance (P3HB) storage. Although high substrate concentrations provided in bulk can suppress polymer accumulation, pulse feeding—particularly with acetate—has been shown to markedly elevate (P3HB) content. Moreover, specific organic acids such as acetate and butyrate have demonstrated stimulatory effects on (P3HB) biosynthesis in mixed photosynthetic cultures, underscoring the potential of tailored feeding strategies to improve both yield and polymer characteristics. Beyond laboratory-scale processes, wastewater treatment plants (WWTPs) provide practical platforms for (P3HB) production, primarily via glycogen-accumulating organisms (GAOs) and phosphorus-accumulating organisms (PAOs). These microbial communities synthesize and mobilize (P3HB)s by consuming carbon sources and glycogen under anaerobic phases, while utilizing phosphate for polyphosphate replenishment and degrading stored (P3HB)s during aerobic phases (Wang et al., 2025).

4.4. pH

Physical growth parameters significantly influence the biopolymer accumulation capacity of microbes. pH levels between 7.0 and 7.5 and an incubation temperature of 30°C are typically optimal for maximum PHB production. A pH of around 7.5 is considered ideal for many microorganisms, as deviations from this range reduce CO_2 absorption capacity and interfere with RuBisCO enzyme activity, leading to poor cell growth and consequently lower PHB accumulation. In microbial culture systems, cells first channel energy into biomass growth before accumulating storage products like PHB. For instance, acidic pH was found to inhibit PHB accumulation in Bacillus cereus, with a maximum optical density (OD) of 0.05 at pH 3.0, compared to an OD of 5.9 at pH 6.8, which resulted in 23% CDW PHB(Valentino et al., 2017). Similarly, the highest PHB accumulation in Pseudodonghicola xiamenensis occurred at pH 7.5–8.0, and for Bacillus sp., PHB production was highest at pH 7–9(da Silva Moura et al., 2019). To address cultivation issues like contamination, pH is often raised to saline concentrations, but this can lead to decreased lipid content and increased carbohydrate content(Feng et al., 2023).

5. EFFECT OF EXTRACTION METHOD ON THE YIELD AND PURITY OF (P3HB)

Microorganisms capable of synthesizing poly(3-hydroxybutyrate) (P3HB) may accumulate the polymer to nearly 90% of their cell dry weight. Despite this high intracellular concentration, efficient recovery remains a major challenge, as downstream operations—particularly harvesting and extraction—can represent 60–80% of the overall production cost (Wang et al., 2025). Consequently, optimizing extraction strategies is essential for achieving high yields while preserving the polymer's structural and functional integrity. Conventional recovery methods include surfactant–chelate systems, solvent-based extraction, mechanical disruption, chemical treatments, and enzymatic processes. Over time, diverse solvents and disruption techniques have been refined to enhance cell lysis and facilitate the release of intracellular polymers (Table 2).

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Table 2. Effect of Various Extraction Methods on the Yield and Purity of (P3HB)

Method	Microbial strain	Extraction condition	yield (%)	Purity (%	References
Organic solvent extraction	Cupriavidus necator	Acetone/ethanol/propylene carbonate (A/E/P, 1:1:1 v/v/v)	83–92%	92–93	(Fei et al., 2016)
	Cupriavidus necator H16	Cyclohexanone, 120 °C 3 min	82.3%	95	(Jiang et al., 2018)
	Spirulina sp.	Sodium hypochlorite, methanol, chloroform, acetone	6.1–9.8	63.51 and 93.62	(Monshupanee and Incharoensakdi, 2014)
	Cupriavidus necator strain A-04	1,3-dioxolane (95, 80, and 85% (v/v)), screw-capped test tubes, 80 °C, 6 h, vortex every 30 min	76.1	97.9	(Jiang et al., 2018)(Fei et al., 2016)
	Nostoc muscorum NCCU-442	Pre-treatment of biomass with methanol:acetone: water:dimethylformamide [40:40:18:2 (MAD-I)] with 2 h magnetic bar stirring followed by 30 h continuous chloroform soxhlet extraction	NaCl and P deficiency yielded 26.37% (P3HB)	N/A	(Ansari and Fatma, 2016)
Enzyme extraction	Pseudomonas putida Bet001	Sonication and heptane (marginal non-solvent)	29.5/37.1%	N/A	(Ishak et al., 2015)
	Cupriavidus necator strain A-04	Ultrasonication, 1,3-dioxolane, 10 min	31.8	73.9	(Jiang et al., 2018)
	Bacillus flexus	SDS sonication	96.7	≥96	(Arikawa et al., 2017)
	Cupriavidus necator	CelumaxVR BC	93.2	94	(Kapritchkoff et al., 2006)
	Ralstonia eutropha DSM545	Bromelain (2%), temperature: 50 °C, pH: 9.0	61.3	88.8	160]
Bioextraction Bacteria extraction system	Pseudomonas putida	Bdellovibrio bacteriovorus (mutant strain)	0.65–0.87 g/L	N/A	(Martínez et al., 2016)
Phage lysis system	Pseudomonas oleovorans	Bacteriophage Ke14	0.84 g/L	N/A	(Hand et al., 2016)
Animal digestive system	Cupriavidus necator	Yellow mealworm (Tenebrio molitor)	55 wt% - from palm olein 60 wt% - from waste animal fats	94	(Ong et al., 2018)

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6. METHODS OF PURIFICATION (P3HB)

Purification of the extracted biopolymer represents a decisive stage in the production process, ensuring the removal of residual impurities such as microbial debris, pigments, odors, and solvent traces following extraction from the cell biomass. This step is particularly crucial in applications demanding high polymer purity, especially in biomedical and pharmaceutical fields. Typical purification strategies include the application of chelating or nutrient-based treatments, often complemented by oxidizing agents such as hydrogen peroxide and ozone. Among these, ozone has recently attracted increasing interest as a viable alternative to hydrogen peroxide, owing to its multifunctional properties in bleaching, deodorization, and effective impurity removal (Feng et al., 2023). The optimization of purification protocols is influenced by multiple factors, including the microbial strain employed for (P3HB) biosynthesis, the degree of purity required, the intended industrial application, and the structural characteristics of the polymer. A promising approach for improving (P3HB) purity while maintaining high recovery efficiency involves ammonia-based treatment at elevated temperatures, which has demonstrated effective performance(Wang et al., 2025).

7. COST-EFFECTIVE METHOD SOLUTIONS FOR DEVELOPING COUNTRIES

The findings of this review provide valuable insights for developing countries, where waste management systems are often limited and cost-effective, sustainable raw materials are urgently needed. Utilizing locally available wastewater (WW) as a low-cost substrate for (P3HB) production presents an environmentally friendly alternative to conventional plastics. Moreover, integrating microbial fermentation with innovative, scalable, and eco-conscious extraction methods—such as two-phase aqueous extraction and removal of non-(P3HB) biomass using green solvents like ethylene carbonate—can substantially reduce production costs while minimizing environmental impact. This approach promotes waste valorization, reduces dependency on expensive raw materials, and creates new economic opportunities. In resource-constrained regions, widespread implementation of such technologies can strengthen local industries, support a circular economy, and mitigate environmental pollution.

8. FOR FUTURE RESEARCH

The future of (P3HB) production, especially via the use of municipal and industrial wastewater, appears highly promising. As a sustainable alternative to conventional plastics, (P3HB) offers thermoplasticity, water resistance, and biodegradability. However, high production costs—mainly due to the need for purified substrates—remain a major limitation for commercial adoption. Utilizing wastewater, rich in organic carbon and nutrients, as a low-cost and renewable feedstock provides a viable solution. Future research should focus on optimizing cultivation and operational parameters, selecting microbial strains that perform efficiently under non-sterile conditions, and improving processing technologies to lower costs while enhancing (P3HB) properties.

9. MAIN LIMITATIONS OF THIS STUDY

This study highlights several critical challenges in (P3HB) production. A primary limitation is the high production cost, largely driven by the expenses of organic carbon sources and essential salts, which substantially affect the economic feasibility of large-scale industrial production. Additionally, extracting (P3HB) from microbial cells remains technically complex and costly due to the structural resilience of the cell walls. While the use of wastewater as a carbon source presents a promising, sustainable, and cost-effective alternative, its success depends on the careful selection of suitable microbial strains and the precise optimization of environmental and culture conditions. Furthermore, certain (P3HB)-producing microorganisms require highly specific growth parameters that may be difficult to replicate at an industrial scale. Although (P3HB) is biodegradable, its degradation under natural conditions can be relatively slow, which may limit its applicability in certain contexts. These challenges emphasize the need for ongoing research to improve production efficiency and to develop economically feasible and environmentally sustainable strategies for (P3HB) manufacturing.

10. CONCLUSION

Studies have shown that substrates rich in volatile fatty acids (VFAs) are highly suitable for (P3HB) production in bacteria. Microalgae, such as Chlorella sp., also demonstrate a strong tolerance to VFAs, making waste streams like anaerobic digester liquors, which are high in VFAs, excellent candidates for (P3HB) biosynthesis in both microalgae and mixed microbial cultures. Utilizing microorganisms cultivated in wastewater for biopolymer production represents a sustainable strategy that combines effective

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wastewater treatment with the generation of biodegradable plastics. The widespread issue of single-use plastic pollution can be addressed by employing mixed microbial cultures capable of growing in wastewater, thereby producing (P3HB) in an eco-friendly manner. Additionally, autotrophic microalgae can exploit inorganic carbon sources, such as CO_2 from industrial flue gases, contributing to both cost reduction and improved effluent quality. Integrating organic-rich waste streams for simultaneous (P3HB) production and wastewater remediation offers a promising approach for future commercial biopolymer production. Implementing a two-stage cultivation system with metabolically versatile microbes, particularly under variable growth conditions, could further enhance production efficiency and economic feasibility.

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