Biodegradable Polymers, Blends and Biocomposites

Trends and Applications

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Chapter 5
Microbial Production of Biopolymers

Recent Advancements and Their Applications

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5 Microbial Production of Biopolymers

Recent Advancements and Their Applications

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5.1 INTRODUCTION

Polymers derived from petrochemical industries are used to manufacture most plastic products. Owing to their resilience and malleability, these petrochemical-based polymers are employed in various industrial applications, particularly in packaging, medical, transportation, and agriculture (Lambert, 2015). Modern petrochemical plastics have many environmental problems, particularly in waste management and the toxic compounds released into the environment in due process. The plastic material has endured for thousands of years and is difficult to break down into more specific, less toxic components by the environmental elements. In addition to the rising use of plastic, the reliance on crude oil has also increased. Toxic compounds and microplastics are leftover during recycling, which also poses a more significant threat to the environment. Microplastics are easily entrapped into the human and animal food chains, which eventually end up in their biological system. Plastics are susceptible to solar radiation, which produces greenhouse gases (GHGs) such as methane and ethylene. According to Lambert (2015), plastic is widely used in various industries such as 40.1% of packaging, 20.4% of construction, 5.6%–7.0% of automotive electrical and electronic equipment, and 26.9% of agricultural equipment.

Nowadays, society strives for sustainable development, leading to the search for plastic substitutes. The production of biodegradable materials from biological resources is known as "biopolymers" or "biobased polymers", and it is a potential solution for waste management (Lambert, 2015). Biopolymers with at least one monomer derived from renewable sources will mitigate environmental issues (J. W. Lee et al., 2011; Vroman & Tighzert, 2009). Polyhydroxyalkanoates, PLA, cellulose, xanthan, dextran, pullulan, starch and cellulose are examples of biopolymers (Lambert, 2015). Biopolymers are not a new notion; bio-cellulose was created in the 1850s.

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In the 20th century, Henry Ford Motor Company experimented with several biopolymers (soy proteins) as an alternative for automotive parts production (Grujić et al., 2017). The oil crisis in the 1970s sparked the importance of biopolymers in the USA. In the 1980s, biodegradable films, sheets, and moulding materials were accessible. Due to the increased plastic pollution and climate change, biopolymers are considered an important alternative to synthetic polymers. Biopolymers have properties similar to petroleum-based polymers, and there are many different forms of biopolymers on the market, including PLA, polyethylene (PE), starch, chitosan, pectin, collagen, polytrimethylene terephthalate, polyp-phenylene succinate (PBS), gelatin, caseins, zein, natural waxes, and microbial-based polymers like polyhydroxyalkanoates. Various biomaterials, including those mentioned above, have been packaging a wide range of food products since long ago. As a result of their mechanical and chemical properties, these biopolymers are gaining traction in food packaging. Global bioplastic manufacturing capacity will rise substantially from around 2.41 million tons in 2021 to 7.59 million tons in 2026 (European Bioplastics, 2021). The high demand for biopolymers is utilised in various applications like packaging services, including both rigid (cosmetics packaging of creams and lipsticks as well as beverage bottles) and flexible (single-use packaging plastics), consumer goods, fibres, agriculture and horticulture, automotive and transport, coating and adhesives, building and construction, consumer electronics and others. Polyhydroxyalkanoates (PHAs) and polylactide (PLA) are the most extensively produced biodegradable polymers for various industrial purposes.

5.2 INTRODUCTION: HISTORY IN THE DEVELOPMENT OF POLY HYDROXYL ALKANOATES (PHAS)

Petrochemical-based polymers are one of the most significant discoveries in the history of humanity and have been used in numerous industries like packaging, transport, automobile parts, agriculture and surgical and medical devices. The overproduction of petrochemical-based plastics has engendered huge complications for terrestrial and marine environments. The plastic is disposed of in two worst modes: environmental dumping and incineration. Dumping plastic from landfills into the domain has been persistent for several years. That does not degrade and remains in the environment for a consistent time, causing land and marine pollution.

Similarly, incineration of plastic waste also releases toxins into the atmosphere, causing hazardous threats to the environment (Nehra et al., 2017). Recently, modern society has recognised plastic as a significant environmental pollutant, and the search for natural alternatives for synthetic polymers has reached an all-time high. Bioplastic has recently been popular for various applications as an alternative to synthetic polymers obtained from diverse plants or microbial sources (Mohanrasu et al., 2021, Rasu and Arun, 2017; Muniyasamy et al., 2019; Muniyasamy and Dada, 2021).

Compared to all biopolymers, microbial-based biopolymers such as polyhydroxyalkanoates (PHAs) are one of the promising alternatives for petroleum-derived polymers owing to their biodegradability and biocompatibility (Kulkarni et al., 2010). PHAs (polyesters) are energy-storage compounds synthesized by various microbes (Gram-negative and Gram-positive bacteria) under nutrient-stress conditions (excessive carbon and limitation of nitrogen or phosphorus) as accumulated granules inside the cytoplasm. PHAs contain various side chains of hydroxyalkanoates (HAs), and around 150 types of (R)-3-hydroxy fatty acids were identified in the PHA family (Roy & Visakh, 2014). Polyhydroxybutyrate (PHB) was first identified in 1926 by Maurice Lemoige from *Bacillus megaterium* bacterium; it is one of the well-studied groups of PHAs with 80% of polymer in total cell dry weight (Keshavarz & Roy, 2010; Yu & Stahl, 2008). Maurice identified lipid-like insoluble material, which was reported in a French journal; moreover, between 1923 and 1951, he published 27 articles reporting a wide variety of microorganisms producing PHB (Chodak, 2008). According to Wilkinson Macre, increased carbon sources (glucose) with nitrogen sources lead to increased formation of granules in the *B. megaterium* (Alves et al., 2017). Subsequently, various microorganisms have been reported to produce P(3HB), such as *Azotobacter*, *Chromatium*, *Hydrogenomonas*, and *Pseudomonas,* between 1959 and 1973 (Braunegg et al., 1998). Wallen and Rohwedder (1974) reported activated sludge as a carbon source for PHA synthesis, followed by numerous waste sources as carbon sources for PHA synthesis. In the 1990s, *Alcaligenes eutrophus* was reported to accumulate 75% of PHB in total cell dry weight that was later renamed *Ralstonia eutropha,* which is one of the potential bacteria for higher PHB production using different carbon sources (B. S. Kim et al., 1994; Wallen & Rohwedder, 1974).

5.3 STRUCTURES AND CLASSIFICATION OF PHA

PHA polymers are structurally classified into three types based on the number of carbon atoms in the monomeric unit with small chain length (*scl*) PHA polymer that ranges from 3 to 5 (C3–C5) carbon atoms, medium chain length (*mcl*) – PHAs ranges from 6 to 14 (C6–C14) carbon atoms and long-chain length (*lcl*) PHAs which poses greater than 14 (>C14) carbon atoms (Pradhan et al., 2020). The *scl* (3–5 carbons) and *mcl* (6–14 carbons) are blended to form copolymers *scl*-co-*mcl* (3–14 carbons); similarly, the *lcl*-PHAs blending was also studied (Steinbuchel and Valentin, 1995; Steinbüchel and Hein, 2001). Short-chain length PHAs polymer includes poly(3-hydroxybutyrate) P(3HB), poly(4-hydroxybutyrate) P(4HB) and poly(3-hydroxyvalerate) P(3HV) or the copolymer P(3HB-co-3HV), Medium-chain lengths PHAs includes 3-hydroxyhexanoate (3HHx), 3-hydroxyheptanoate (3HHp), 3-hydroxyoctanoate (3HO), and 3-hydroxydecanoate (3HDD) (Anderson et al., 1992).

The PHA synthase substrate specificity is the main reason for the varying carbon length of 3HAs. According to Khanna and Srivastava (2005), *A. eutrophus* PHA synthase can polymerise 3–5 carbon atoms of 3HAs, but *Pseudomonas oleovorans* PHA synthase polymerises 6–14 carbon atoms of 3HAs. Both short-chain and medium-chain monomeric units are linked to form hybrid polymers like poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); these polymer formations are caused by the biosynthetic enzyme's stereospecificity with R-configuration in all monomers. The monomeric units have various functional groups like carboxyl, esterified carboxyl, cyano, epoxy, halogen and hydroxyl (Castilho et al., 2009; Ciesielski et al., 2015). Among the PHAs, *scl* groups with four carbon atoms homopolymer (Poly 3-hydroxybutyrate) are industrially first recognised owing to their high melting point of 180°C together with high molecular weight and crystallinity. The *scl* groups homopolymers are highly crystalline and thus easily breakable. In contrast, the copolymers have reduced the brittleness and crystallinity (20%–40%), but using a medium chain length, PHAs with their copolymers do not break easily (Anderson & Dawes, 1990).

Among the PHAs, PHB is a well-characterised polymer with a highly crystalline nature because of stereoregularity; moreover, PHB is insoluble in water and resistant to hydrolytic degradation (Anjum et al., 2016). PHB has good thermoplastic properties with poor mechanical properties such as low tensile strength and Young's modulus with low O_2 permeability than petroleum-based polymers (Sudesh et al., 2000). The densities of amorphous and crystalline PHBs are 1.18 and 1.26 g/cm^{3,} respectively, with the molecular weight in the range of 10,000–3,000,000 Da from wild-type bacteria (Anjum et al., 2016). Poly(4-hydroxybutyrate) P(4HB) properties (thermoplastic and tensile strength) are like synthetic polyethylene, which has extreme elastic properties. The copolymer produced from combining any two or more PHB such as 3-hydroxyvalerate, 3-hydroxyhexanoate, 3-hydroxypropionate and 4-hydroxybutyrate improves the material properties like crystallinity, melting point, stiffness and toughness. The P (3HB-co-3HV) is the most investigated copolymer with decreased stiffness, lower melting temperature, and lower crystallinity, but it is flexible and more challenging (Anjum et al., 2016). On the other hand, terpolymer has better desirable properties than copolymers like P (3HB-co-3HV-co-3HHx).

5.4 BIOSYNTHESIS OF PHAS

PHAs are biodegradable polymers produced by several (above 300) gram-positive and gram-negative bacteria inside the cytoplasm under excessive carbon and limited nitrogen or phosphorus conditions. The PHB granules are made inside the cytoplasm; about 0.2 ± 0.5 mm in diameter can be visualised by staining dyes such as Sudan Black B, oxazine dye Nile Blue A/Nile red under phase contrast light microscope due to the high refractivity index (Anjum et al., 2016). The synthesis of PHA is a complex process regulated by many genes that encode a wide range of enzymes that are directly or indirectly involved in PHA synthesis (Laycock et al., 2013). Numerous bacteria are isolated from marine and terrestrial environments for higher PHA-producing capability, and several strains showed enhanced PHA production from marine and terrestrial environments (Mohanrasu and Arun, 2017; Pradhan et al., 2020; Mohanrasu et al., 2021).

PHA production involved in eight pathways is summarised in *Ralstonia eutropha* as a model organism. The PHA production pathway I involved three enzymes: β-Keto thiolase, NADPH-dependent acetoacetyl-CoA reductase and PHA synthase. These enzymes are encoded by phaA, phaB and phaC genes (Laycock et al., 2013). Pathway II is involved in fatty acid uptake, β oxidation from acyl-CoA, 3-hydroxyacyl-CoA, and PHA production by synthase catalysis. Pathway III involves 3-hydroxyacyl-ACP-CoA transferase, malonyl-CoA-ACP transacylase (FabD) encoded by PhaG and substrates converted 3-hydroxyacyl-ACP to 3-hydroxyacyl-CoA and PHA. Pathway IV involves NADPH-dependent acetoacetyl-CoA reductase to oxidise

(S)-(+)-3-hydroxybutyryl-CoA. The remaining pathways are involved in copolymer production; P(4HB) production is done by pathways V and VII in *Clostridium kluyveri* and *A. hydrophila* 4AK4 (Laycock et al., 2013).

5.5 SUBSTRATES FOR PHAS PRODUCTION

The main problem associated with PHB production is higher production costs. The selection of carbon sources and strains enhances PHB production and is cost-effective. The choice of appropriate carbon sources for PHA production, such as simple sugars or industrial, agricultural, and food waste, has proved to be successful in enhancing PHA production (Table 5.1). In the initial stages of PHA production, simple sugars are mainly used as carbon sources for PHA biosynthesis compared to waste sources. The structure of synthesized PHAs is strongly impacted by various carbon sources and different microbes used; this structural composition influences the applications of PHA. The *Pseudomonas* species producing PHA has other functional groups (phenyl, phenoxy, halogens, branched alkyls, olefin and esters) based on the substrates utilised (Pradhan et al., 2020). Kim et al. (2011) analysed 36 different carboxylic acids containing carbon substrates for PHA production employing *Pseudomonas putida* KCTC 2407 to obtain an appropriate functional group into the PHA chain to enhance the physical properties (Pradhan et al., 2020). The carbohydrates are categorised into three types such as monosaccharides, disaccharides, and polysaccharides; monosaccharides are simple sugars that bacteria can efficiently utilise for PHA production. The disaccharides and polysaccharides cannot be directly used; instead, they should be hydrolysed and converted into monosaccharides for PHA production by bacteria (Pradhan et al., 2020).

Several studies reported utilising glucose as a carbon source for PHA production. Even though many researchers have examined different carbon sources (arabinose, glucose, glycerol, lactose, lactic acid, mannitol, sodium acetate, starch and sucrose) for PHA production, most found glucose to be the optimum carbon source for maximum PHA production of 5.61 g/L by *Bacillus megaterium* (Mohanrasu et al., 2020). Arun et al. (2009) isolated 42 different stains from various marine environments and screened their PHB-producing capabilities. Other carbon sources (arabinose, glucose, glycerol, lactose, lactic acid, mannitol, sodium acetate, starch and sucrose) have been screened for best PHB production; compared to other carbon sources, glucose showed higher (4.223 g/L) PHB production. Obruca (2015) reported better PHA production using sucrose directly as a carbon source using *R. eutropha*. In another study, pure glycerol was found to be the best carbon source for PHA production by recombinant *E. coli* and *Shimwellia blattae* strains with 63% and 30.7% PHA content, respectively (Nikel et al., 2010; Sato et al., 2015).

However, in recent years, waste from different agricultural and industrial sources has been utilised as a potential medium to decrease PHA production costs. For many regions globally, waste sources are explored as a possible factor for converting value-added products. In these studies, various waste sources are used for PHA production, such as lignocellulosic biomasses from wheat straw lignocellulosic hydrolysates (Cesário et al., 2014), oil-palm biomass (Hassan et al., 2013), hydrolysates

TABLE 5.1
Production of PHAs by Bacterial Strains Using a Wide Range of Feedstocks

PHAs Type	Carbon Source	Microbial Strains	Production Scale	PHAs (g/L)	References
Poly(3-hydroxybutyrate) (PHB)	Glucose and glycerol	*C. necator* DSM 545 and *Burkholderia sacchari*	Fed-Batch culture	44.25 and 4.48	Rodríguez-Contreras et al. (2015)
P(3HB)	Crude glycerol, rapeseed waste	*Cupriavidus necator* DSM 545	Fed-batch	10.9	García et al. (2013)
P(3HB)		*C. necator* DSM 428	Batch	0.342	Dhangdhariya et al. (2015)
P(3HB)	Glucose	*Micrococcus luteus* (KY494862)	Shake flask	4.632	Mohanrasu et al. (2018)
P(3HB-co-3HV)	Crude glycerol	*Haloferax mediterranei* DSM1411	Fed-batch	15.2	Hermann-Krauss et al. (2013)
P(3HB-co-3HV)	Synthetic wastewater	*Hydrogenophaga palleronii* NBRC102513	Batch	1.01	Venkateswar Reddy et al. (2016)
P(3HB)	Glucose	*Vibrio* sp. (MK4)	Batch fermentation	4.223	Arun et al. (2009)
P(3HB)	Glucose	*Micrococcus luteus* (KY494862)	Batch fermentation	5.61	Mohanrasu et al. (2020)
P(3HB-co-4HB)	Waste corn-steep liquor, gluconate	*Halomonas bluephagenesis* TD01	Fed-batch	66.6	Ye et al. (2018)
P(3HB-co-3HHx)	Crude glycerol	*Ralstonia eutropha* Re2133	Batch	0.57	Bhatia et al. (2018)
P(3HB)	Methanol	*Methylobacterium extorquens* DSMZ 1340	Batch	9.5	Mokhtari-Hosseini et al. (2009)
PHBV	Pre-treated vinasse	*Haloferax mediterranei*	Shake-flasks	19.7	Bhattacharyya et al. (2012)
P(3HB)	Commercial glycerol	*C. necator* DSM 545	Fed-batch	51.2	Cavalheiro et al. (2009)
P(3HB)	Waste glycerol	*C. necator* DSM 545	Fed-batch	38.1	Cavalheiro et al. (2009)
P(3HB)	Glucose	*Bacillus cereus* (KR809374)	Fed-batch	19.15	Dinesh et al. (2020)
P(3HB)	Glucose	*Micrococcus luteus*	Fed-batch	12.18	Mohanrasu et al. (2021)

of spruce sawdust paper waste, wood chips (D.-H. Kim et al., 2011), paddy straw (Sandhya et al., 2013), corn cob, corn stalks, eucalyptus, pine, sugarcane bagasse, sorghum straw, oat straw, barley hull and grasses (Kucera et al., 2017).

Apart from lignocellulosic biomasses, extensive research is done on different agricultural and food wastes such as waste rapeseed oil (Stanislav Obruca et al., 2010), jatropha oil (Ng et al., 2011), coprah oil (Simon-Colin et al., 2008), linseed oil (Bassas et al., 2008), corn oil (Chaudhry et al., 2011), municipal waste waters (Chua et al., 2003), waste sesame oil (Arun et al., 2006), waste potato starch (Haas et al., 2008), waste cooking oil (Haba et al., 2007), waste frying oil (Verlinden et al., 2011), palm oil mill effluents (Wu et al., 2009), brewery waste effluents (Liu et al., 2011), kraft mill waste water (Pozo et al., 2011), biodiesel waste water (Dobroth et al., 2011), alpechin medium (waste water from olive oil mil) (Ntaikou et al., 2009), food processing waste effluents (Reddy et al., 2017) and potato processing waste for PHA production (Rusendi & Sheppard, 1995).

5.6 THE CURRENT SCENARIO FOR INDUSTRIAL-LEVEL PHA PRODUCTION

Among different types of PHA, only four different varieties are gaining industrial importance such as poly[(*R*)-3-hydroxybutyrate] (PHB), poly[(*R*)-3-hydroxybutyrate-*co*-4-hydroxybutyrate] (P3HB4HB), poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyvalerate] (PHBV) and poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyhexanoate] (PHBHHx) (Chen, 2010). PHA production depends upon several processes such as isolation of the best possible strains from wild or recombinant sources, optimisation of numerous parameters influencing the production, scaling up the output to industrial-level fermentation, biomass drying, extraction, and purification of PHA. Currently, 24 companies are known to produce PHAs globally, but some of them have stopped PHA production due to the rise in the cost of production (Chen, 2010).

5.6.1 Metabolix

Metabolix company has produced PHA for the longest time aimed at commercial applications. This company has more than 500 patents for diverse industrial applications. PHA is made in the name of Mirel, which rapidly reached 50,000 tons production capacity a construction facility (Chen, 2010).

5.6.2 Biopol

Biopol is an Imperial Chemical Industries (ICI), UK, the company that sold its patents to Zeneca, which was brought by Monsanto and currently produces in the name of Metabolix. Biopol mainly had a wide range of products like packaging materials, medical, surgical pins, disposable knives and forks, disposable cups, shampoo bottles and disposable razors using copolymers poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (Anjum et al., 2016).

5.6.3 Nodax

Nodax also produces a copolymer of 3-hydroxybutyrate with medium chain length monomers, including 3-hydroxy hexanoate, 3-hydroxy octanoate and 3-hydroxydecanoate. Nodax has films, latex and fibres or non-wovens.

5.6.4 Biogreen

Biogreen is a Japanese-based company (Mitsubishi Gas Chemicals) that produces P (3HB) from methanol. Biogreen produced various industrially essential materials such as cosmetics containers, packaging, shampoo bottles, cups, milk cartons, diapers, feminine hygiene products, sanitary towels, food additives, sanitary towels, mulch films, herbicides, insecticides, bacterial inoculants, cardiovascular stents and heart valves, vascular grafts, surgical sutures, dusting powders, wound dressings, nerve conduits, bone plates and osteosynthetic materials (Anjum et al., 2016).

5.7 BIODEGRADABILITY AND BIOCOMPATIBILITY OF PHAS

A microorganism degrades complex macromolecules through the action of their enzymes into simpler small molecules, known as biodegradation. Biodegradation (aerobically or anaerobically) is every biopolymer's most desired and essential feature. Moreover, the degraded product should not produce harmful compounds or toxins in the environment. PHAs can be degraded entirely under aerobic and anaerobic conditions; during degradation, they break down into carbon dioxide and methane in soil, lakes, sewage, and marine environments by microorganisms. The incredible feature of PHA is that when disposed of the material environments, the diverse microbes prevailing in the habitat secrete depolymerase enzyme, which hydrolyses the PHA into water-soluble oligomers and monomers that can be efficiently utilised as nutrients for the growth of the microbes (Schneider et al., 2010).

The PHA can be degraded in a wide range of environmental habitats like marine and terrestrial ecosystems; the PHA degradation is influenced by several external environmental factors such as pH, temperature, a load of microbes in the particular environment, humidity and molecular weight of the polymer (Khanna & Srivastava, 2006). Apart from external factors, PHA characteristics such as stereospecificity, crystallinity, molecular weight, melting temperature, and monomeric composition of PHAs also strongly impact PHA degradation (Jendrossek et al., 1996). Several researchers have explored the potential degradation of PHA in terrestrial and marine environments; in the terrestrial environment, 85% of the PHA can be degraded within seven weeks. In aquatic conditions, it requires 254 days to degrade PHAs, but the only limitation is that the temperature should not exceed 60°C (Anjum et al., 2016). The bacterial family such as Micromonosporaceae, Streptosporangiaceae and Streptomycetaceae, Pseudonocardiaceae can degrade P (3HB) in the environment (Anjum et al., 2016).

5.8 PHAS AND THEIR APPLICATIONS IN DIFFERENT SECTORS

The research towards PHA productions has spotlighted the potential owing to its valuable properties in diverse applications such as biomedical, packaging materials, industrial, and agricultural sectors biomedical, packaging materials, industrial, and agricultural applications. The PHA characteristics, including solubility in water, optically active nature, and crystalline and piezoelectricity, make it an attractive material. The significant attributes of PHA are non-toxic, biodegradable, and biocompatible materials compared to other synthetic polymers such as polypropylene (PP) and polyethylene (PE) (Nehra et al., 2017).

5.8.1 Industrial Sector

PHA is used mainly as an alternative to synthetic polymers for numerous industrial applications. The US-based company produced blends of P(3HB) and P(3HO); moreover, the FDA has approved these polymers' usage for food packaging sectors (Rai & Roy, 2011). PHAs from *Aeromonas hydrophila* were produced on an industrial scale by a collaboration of KAIST (Korea) and Procter & Gamble (P&G, USA), where the company made various critical industrial products such as flexible packaging, flushable, non-wovens, thermoformed articles, binders, synthetic paper and medical devices (Rai & Roy, 2011). A German company produced P(3HB) biomer by using *Alcaligene latus* to make innovative materials like pens and combs (Chen & Wu, 2005). BIOPOL® also manufactured a copolymer of P(3HB-co-3HV) for packaging, fishing nets, paper boards, electrical appliances, ropes, preparation of coat papers and storing containers like shampoo razors and motor oil (Pradhan et al., 2020). The copolymers *mcl*-PHA and P(3HB) synthesised by the Nodax™ company were used for the films and foam production (Philip et al., 2007).

In general, plastics are used in food sectors to extend their shelf life by avoiding contact with microorganisms, thus reducing the risk of food spoilage, restricting the moisture and light and maintaining the food texture, retaining the flavours of food by preventing connection with any of the following substances such as oxygen, carbon dioxide, water vapour and volatile compounds. On the contrary, in recent years, PHAs have been an alternative to synthetic polymers for food packaging applications because of their qualities like biodegradability, environmentally friendly and renewable nature. The perishable foods (raw meat, cured meat and meat salad) packaging is done by PHAs to reduce municipal solid wastes. It is also used in various food sectors such as fresh fruit packaging, dairy product packaging, medicine packaging and fragile item packaging (Mudenur et al., 2019).

5.8.2 Medical Sector

Being of bacterial origin, PHAs are extensively used in the medical fields, such as scaffolds in the form of screws, pins, cartilage repair, PHB ultrafine fibres for skin regeneration, bone tissue regeneration, articular cartilage, cardiovascular patch grafting and meniscus repair devices (Ray & Kalia, 2017). Moreover, PHAs are also utilised in stem cell growth, as a vehicle for targeted drug delivery, and as raw material for various moulded products such as syringes, sutures, disposable needles, surgical gloves and gowns (Ray & Kalia, 2017). The targeted drug delivery system with a controlled optimal delivery rate and dose in a specific targeted site is the fascinating application of PHA in biomedical fields. As the polymer is biocompatible and degradable, it is utilised as a drug delivery system both orally and intravenously.

5.8.3 Agricultural Sector

In agricultural fields, PHA nanocomposites are modified like plastic mulch to be used over the surface of the land to prevent weed growth, protect the vital ingredients in soil like nutrients, and escape the soil through evaporation. These types of plastic mulch are exceptionally eco-friendly, produced from renewable sources and reduce labour costs (Ivanov et al., 2015). The PHB used in plant growth-promoting rhizobacteria formulations protects the bacteria from extreme stress conditions like high or low temperatures, desiccation, chemical pesticides and differences in pH during transportation and storage (Benrebah et al., 2007). The PHA acts as transporting materials due to its properties such as lack of toxicity, chemical and physical uniformity, high water-holding capacity, and eco-friendly material used in powder and liquid formulations (Stephens & Rask, 2000). On the other hand, PHAs are also used as a carrier for insecticides that will release the insecticide into the soil while facing specific environmental conditions (Holmes, 1985; Philip et al., 2007)

5.9 LIMITATIONS AND RECENT ADVANCES IN PHAS PRODUCTION AND MARKETING

1. The most significant problem associated with PHA production is the high production cost compared to petroleum-based polymers. Exploiting new application fields should reduce the cost problems.
2. The PHA production costs are 15 times higher than the petroleum-based polymer, which could be credited to using carbon sources such as arabinose, glucose, glycerol, lactose, lactic acid, mannitol, and sodium acetate, starch, and sucrose as the cost of these chemicals are not stable.
3. Therefore, PHA production costs can be considerably lowered using industrial byproducts such as wastewaters, agriculture feedstocks, waste plant oils and kitchen wastes.
4. Another significant limiting factor of PHA production is the extraction process which consumes around 50% of the total production cost and requires various chemical digestion and solvent extraction; moreover, this leads to environmental problems with socio-economic losses.
5. The bioextraction techniques (genetic and metabolic engineering) can be a fantastic alternative for extraction by solvents (hazardous to the environment). These techniques include mealworm systems, bacteriophage-mediated lysis and predatory bacteria.

6. Industrial-scale PHA production with selected appropriate strains should be based on fast-growing capabilities, higher PHA production ability, a wide range of substrate utilisation, salt concentration, temperature and ease of genetic modification. Finally, strain should not produce toxins for various industrial applications.
7. The PHAs blended with different HA monomers such as poly(4-hydroxybutyrate) P(4HB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) P(3HB-co-3HV), P(3HB-co-3HV-co-3HHx), and blend with natural polymers like poly(3-hydroxybutyrate) (PHB)/chitin blends, poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV)/chitosan, poly(3-hydroxybutyrate)/cellulose acetate butyrate PHB/CAB, poly(hydroxybutyrate)/cellulose propionate (PHB/CP), PHB/hydroxyethyl cellulose acetate (HECA) and different PHA nanocomposites have been investigated in various studies for maintaining the outstanding properties and wide industrial application.
8. Despite the existing hurdles, the strategies mentioned above would undoubtedly improve the PHA's production and will positively impact various industrial applications, which would eventually play a vital role in different outfronts shortly.

5.10 BACTERIAL CELLULOSE (BC)

In 1886, bacterial cellulose gained attention due to its unique properties such as biodegradability, biocompatibility, high purity, good chemical stability, higher mechanical strength, crystalline network structure, and better water-holding capacity (Santos et al., 2015). Cellulose is the most abundant, sustainable, and widely used polysaccharide on the planet, and it is one of the most commonly used polysaccharides (Ullah et al., 2016). In terms of long-term development, bacterial cells can produce BC for a broad range of industrial products. It is a semicrystalline polysaccharide composed of linear homopolymers of D-glucopyranose residues ($C_6H_{11}O_5$) linked together by b-(1 → 3)-glycosidic bonds. BC is produced by various bacteria, including *Acetobacter*, *Pseudomonas*, *Sarcina*, and *Gluconacetobacter* (Pandit & Kumar, 2021).

Several parameters have been optimised to maximise BC yield, regardless of whether the cultivation is agitated or static. pH, dissolved oxygen, and temperature are examples of these parameters (K.-Y. Lee et al., 2014). On an industrial scale, BC manufacturing necessitates a continuous or semicontinuous process, low-cost raw ingredients, and little byproduct generation. It also necessitates a high carbon conversion rate to cellulose and a high speed of BC synthesis (Çakar et al., 2014). BC's exceptional qualities make it ideal for various applications, including transparent food packaging, water treatment, battery separators, adsorbents, tissue engineering scaffolds, artificial blood vessels, and electric conductors or magnetic materials. BC must overcome some technical challenges before reaching an industrial scale. Its hydrophobicity, limited solubility, and relatively high cost are among them. Improving cultivation methods through genetic engineering approaches aids in problem resolution. The primary goals of growing BC studies are modifying BC residences and yields, reducing manufacturing expenses, and selecting appropriate business fabrication methods.

5.10.1 Polylactic Acid (PLA)

In recent years, there has been a significant increase in the production of commercial microbial products. Due to the growing global energy and environmental challenges, researchers have been developing green production methods for nearly decades. PLA has gotten increasing attention due to its eco-friendly approaches and use in packaging, pharmaceuticals, and cosmetics. The ring-opening polymerisation (ROP) and direct polycondensation routes are used to convert lactic acid monomer into PLA (Sin et al., 2013a). Lactic acid (LA) comprises three carbon a-hydroxycarboxylic acids found in two stereoisomers: D-LA and L-LA. PLA also has significant market worth because of its biodegradable and biocompatible qualities. LA is produced from a range of carbon sources and then turned into PLA. LA can be synthesised in two ways: chemically or by fermentation.

Compared to chemical synthesis, the fermentative method of PLA production has several advantages, including optically pure D- or L-LA, low energy consumption, waste materials as substrates, and low production temperatures with higher LA-producing microbes (Abdel-Rahman et al., 2011). A catalytic ROP technique converts the dimers of L-lactide, D-lactide, and meso-lactide to a high-molecular-weight polyester (Sin et al., 2013b). The crystallisation rate, melting point, and mechanical properties are based on the stereochemical composition of PLA (Drumright et al., 2000). PLA demand is increasing due to its transparency, durability, and good mechanical strength compared to other biodegradable polymers. The potential biodegradable and biocompatible properties result in significant market expansion for PLA. In 2019, PLA produced around 190,000 tons worldwide 2019, and a multi-million-ton production will be made in the upcoming decade (Jem & Tan, 2020). According to Djukić-Vuković et al. (2019), 3141 patents were filed (LA and PLA) from 2009 to 2018, which showed that 30.1% (947) are associated with human requirements, particularly foods (502), veterinary science and hygiene (432), and medicine.

LA is synthesised primarily through fermentation using a wide range of materials (both synthetic and waste) and various microbes such as bacteria, fungi, and yeast. LA can produce a wide range of microorganisms, including algae and cyanobacteria (*Hydrodictyon reticulum*, *Scenedesmus obliquus*, *Nannochlorum* sp. 26A4, and *Synechococcus elongates*), bacteria (*Bacillus coagulans*, *Lactococcus*, *Lactobacillus plantarum*, *Carnobacterium*, *Lactococcus lactis*, *Tetragenococcus*, *Pediococcus*, and *Vagococcus*), fungi (*Rhizopus oryzae*, Ceriporiopsis subvermispora, *Mucor* and *Monilia* sp) and yeast (*Saccharomyces, Zygosacchromyces*, and *Kluyveromyces*) (Djukić-Vuković et al., 2019).

The cost of raw materials is one of the most critical elements in economically manufacturing LA. Various renewable alternative materials, such as whey, yoghurt, glycerol, food industry wastes, starchy biomass, lignocellulosic biomass, algal biomass, and agricultural byproducts, are currently underway to reduce the production costs (Abdel-Rahman et al., 2013). Fermentation methods differ depending on higher yields [batch, fed-batch, semicontinuous (repeated batch), and continuous fermentation]. Each has its own set of advantages and disadvantages, such as ease of operation, low contamination risk, time-saving processes, and high productivity, as well as drawbacks such as lower yield, inhibition of end products and incomplete utilisation of carbon sources (Abdel-Rahman et al., 2013). Before starting the fermentation process, we ought to sort out the optimum parameters for fermentation, such as substrate, organic loading rate, carbon/nitrogen ratio, pH, temperature, inoculum concentration, and inoculum age.

Many major companies have joined the PLA sector, including Toyota, Mitsubishi, Mitsui, and Dow, and some others have intentions to launch, such as Uhde, Galactic and COFCO (Jem et al., 2010). Many governments are actively promoting PLA manufacturing as part of an attempt to develop biobased and biodegradable polymers to reduce pollution. PLA production capacity has expanded from less than 100,000 tons to more than 1 million tons in the last 15 years (Jem et al., 2010). The commercial scale of PLA production is lagging due to higher costs compared to synthetic plastics. Several countries, such as France, Korea, Italy and Taiwan, have increased attempts to ban or restrict the use of traditional plastics applications and tax reductions for biobased plastics to remove PLA's economic obstacle (Jem et al., 2010).

To reduce PLA production costs, a fully integrated ROP capacity should exceed 50,000 tons per year. The cost of unit production is high in a small plant, and the competitive edge is low, but the risk of entry increases in a large field (Jem et al., 2010). Apart from the PLA polymerisation part, the industry should focus on upstream LA and lactide production to minimise the economic scale of production. PLA polymerisation plants can be smaller than 50,000 tons per year if the lactide plant is decoupled from the polymerisation plant and still be competitive (Jem et al., 2010). The synthetic plastic manufacturing and incineration processes are emitting CO_2 and are a potential threat to the environment in the form of global warming. Considering PLA's biocompatibility and bio responsibility, it can be used in various biomedical applications, including sutures, implants, fracture fixation, and drug delivery. A sustainable alternative to petrochemical-derived products has emerged due to PLA's biodegradable characteristics and the nature of its feedstock, which makes it an environmentally sustainable choice. The capacity of a fully integrated ROP PLA production facility would reach 50,000 tons per year to lower PLA production costs. In a small unit, the cost of unit production is high, and the competitive advantage is minimal, but in a large one, the risk of entry is significant (Jem et al., 2010). Apart from PLA polymerisation, the industry should concentrate on upstream LA and lactide synthesis to reduce manufacturing costs. If the lactide plant is separated from the polymerisation plant, PLA polymerisation plants can be less than 50,000 tons per year and still be competitive (Jem et al., 2010). The manufacturing and incineration of synthetic plastics emit CO_2, threatening the environment through global warming. Due to its biocompatibility and responsibility, PLA can be employed in various biomedical applications, including sutures, implants, fracture repair, and drug distribution. PLA has developed as a sustainable alternative to petrochemical-derived products due to its biodegradable properties and the nature of its feedstock, making it an environmentally friendly option.

5.11 CHALLENGES AND OPPORTUNITIES

The novel coronavirus (SARS-CoV-2), or COVID-19, has created a pandemic situation around the globe since December 2019 and has significantly affected people from all walks of life. COVID-19 has dramatically increased human transmission anxiety and resulted in the extensive use of single-use plastics in packed foods, groceries and disposable utensils (Mittal et al., 2022). According to the WHO (2020), 89 million face masks, 76 million glove pairs, and 1.6 million pairs of protective goggles are in need every month to meet the rising global demand, and this vast surge is due to the organisation proposed mandates that hospitals should have attire to strictly. During the pandemic's peak, Wuhan (a city in China) alone generated about 240 tons of medical waste per day during the outbreak, almost six times more than it had produced previously (Singh et al., 2020).

The increased plastic waste from the pandemic has posed an increased threat to the globe; thus, biodegradable plastics are a hot topic today. The excellent property of a biodegradable polymer is biodegradation. The biodegradable polymers are converted into CO_2 (aerobic conditions) and CH_4 (anaerobic conditions) in natural environments with the help of natural microflora. The amount of biodegradation (per cent biodegradation) and production (CO_2 or $CO_2 + CH_4$) should be measured. The biodegradable polymer should be 100% biodegradable and not release any harmful toxic materials during the biodegradation process.

The biodegradation process is ensured by various standards methods such as ASTM D6954-04, AS 4736-2006 (American Society for Testing and Materials), ASTM D5209-92, ASTM D5338-98, ASTM D5526-94, ASTM D5951-96, ASTM D5988-03, ASTM D6002-96, ASTM D6340-98, ASTM D6400-99, ASTM D6691-01, ASTM D6692-01, ASTM D7081-05, DIN V 54900-2, EN 13432:2000, EN 14045:2003 (EN 14046:2003, EN 14047:2002, EN 14048:2002, EN 14806:2005, ISO 14851:1999 (International Organization for Standardization), ISO 14852:1999, ISO 14855:1999, ISO 14593:1999, ISO 15314:2004, ISO 16929:2002, ISO 17556:2003, ISO 20200:2004, BS 8472, BS EN ISO 14855-2:2009 (British Standards) and BS EN 13432:2000) (Ammala et al., 2011).

Biopolymer degradation is influenced by various environmental factors such as the nature of the biopolymer, moisture, temperature and pH. In addition, the biodegradation rate is affected by the size of the polymer, crystallinity, copolymer composition and molecular weight (Kale et al., 2007). The biodegradation rate is positively altered by water and moisture; increased moisture and water content result in higher microbial growth and faster degradation (Thakur et al., 2018).

Currently, various countries have taken steps for policies related to bioplastic production. The Malaysian government took the new initiative for Bioeconomy Initiative Malaysia, an initiative to accelerate biotechnology commercialisation (Moshood et al., 2021). This project includes industrial biotechnology, biobased chemical, medical equipment, medical biotechnology, biopharmaceutical, agricultural biotechnology and vaccine manufacturing (Moshood et al., 2021). In China, the National Development and Reform Committee have allocated funds to biodegradable plastics. The biopolymer research undertaken by different Chinese institutes such as the University of Tsinghua, the Chinese Academy of Sciences' Institutes of Physics and Chemistry, and Sichuan University mainly focused on PLA production (Moshood et al., 2021). The Japanese government took the initiative to reduce fossil fuel usage and promote biotechnology biomass to mitigate global warming. Toyota plans to shift 20% of its plastics to bio-sourced plastics by 2015 for its cars (Moshood et al., 2021).

The Korean government reduced the dependability on crude oil through the Industrial Biotechnological Promotion Plan in 2012 (Moshood et al., 2021). Korean-based companies such as LGHausys, SK Chemical, and Hyundai Motors are substituting their petroleum-based material with toxic biopolymers (Moshood et al., 2021). The Korean Bioplastics Association supports the bioplastics supply and biomass authentication. Thailand has over 4000 plastics industry companies, and the nation has a strong biomass industry (Moshood et al., 2021). In Thailand, the bioplastic industry has been formed as a strategic industrial sector for boosting economic growth and sustainability. In the European Green Agreement, there has been a commitment by European countries to transform their linear economies into balanced circular economies by 2050 (Moshood et al., 2021).

The bioplastics industry in India is still in its infancy, and very few companies are involved in the production of bioplastics. In India, nowadays, various aspects such as government funds for bioplastics, Environmental awareness projects, and easy access to feedstock are promoting the growth of bioplastics industries (Mittal et al., 2022). An initial move has been taken, which is banning single-use plastics in various hill stations in India, and scientists are working towards an alternative for bioplastics. Biotechnology-based companies such as Biogreen, Envigreen, 2M Biotech, Plastobags, Truegreen and Ecolife have started bioplastics production in India. United States Department of Agriculture (USDA) has a Bio Preferred program, which initiated the purchase of biobased products and reduced reliance on petroleum-based products (USDA, 2020). The major bioplastics manufacturers have filed U.S. patent (230) for the application of (polyhydroxyalkanoate (PHA), PLA and starch) between 1993 and 2012, such as Novamont, Metabolix, Cargill (Nature Works), Cereplast, Kimberly Clark, Biotec and Nissei (Byun and Kim, 2014).

According to National Research Strategy Bioeconomy 2030: Our Route towards a Biobased Economy is done by Germany, which moves towards the bioplastics alternative for petroleum-based products (BMBF, 2011). The Japanese government announced two programmes, the Biotechnology Strategic Scheme and the Biomass Nippon, whose objective is to promote renewable sources utilisation (Taniguchi, 2018). In Spain, several organisations are promoting bioplastics, such as the Spanish Technological Biomass Platform (BIOPLAT), the Spanish Sustainability Observatory (OSE), the Platform of Biotechnological Markets, and the National Food & Agriculture Forestry Innovation and Research Program to promote linear economy to circular economy (Garrido et al., 2021). There is no official policy on bioplastics in Italy other than banning the distribution of traditional plastic carrier bags in favour of biodegradable bags that have been in effect (Garrido et al., 2021). Although the Canadian government lacks an overarching bioeconomy strategy, it has a series of diverse and often competing policy frameworks and visions (Birch, 2016).

5.12 SUMMARY AND OUTLOOK: A PERSONAL VIEWPOINT

Globally, most of the population is unaware of bioplastics, yet many believe biodegradable plastics are better for the environment than "traditional" plastics. In the designated disposal environment, microorganisms break down the bioplastics safely and efficiently via a microbial food chain. Bioplastic waste disposal must be managed carefully using various methods, such as mechanical or chemical recycling, and it might be better from an economic and environmental standpoint. Biodegradable polymers are frequently promoted in the media. Plastic derived from plants, eco-friendly plastic, and plastic without CO_2 footprint are all gaining significant attention. However, attempting to make this a reality will be challenging.

Our current issues are likely to be remedied by bioplastics, yet such a strategy would be exorbitant and have significant ramifications for our economy and working-class people. Bioplastics have been lauded for their contribution to sustainable living, although most have been regarded from an eco-friendly perspective. Pepsico, for example, has been acclaimed for producing PET from 100% biobased material that is biobased but not biodegradable. Few nations have rules and regulations, a disadvantage in bioplastics manufacturing. Carbon tax increases and stricter government regulations, as well as public opinion, are supported. However, the function of political law will grow in importance.

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