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Review Of Polyhydroxyalkanoates' Trends And Challenges

Abstract

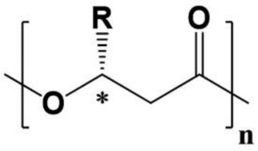
PHAs are promising biopolymers produced from waste, biodegradable and biocompatible, but their synthesis and high cost are still big challenges affecting their mass scale production.

Introduction

As declared by the European Commision in 2018, of the 350 million tons of plastics produced annually worldwide, only 2% is recycled and around 32% goes missing in the ecosystem. In fact, 70% of the plastic waste is transported to landfills or burned in incinerators, were most of them don´t degrade and remain for hundreds of years. PHAs have been proposed to partially replace traditional chemical plastics, including polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), to solve pollution issues posed by nondegradable plastics.

Polyhydroxyalkanoate (PHA) is a biopolyester synthesized by a wide range of prokaryotes and certain eukaryotes. Typically, PHA is accumulated under stressed conditions to serve as an energy and carbon storage material. It is well established that many bacteria and archaea are able to store a significant amount of PHA as intracellular granules within the cytoplasm, when the carbon source is plentiful, but the other nutrients needed for normal cell growth, like nitrogen, phosphate, magnesium or sulfate are lacking. Archaea and various bacterial strains like Gram positive bacteria, Gram negative bacteria, photosynthetic bacteria and mixture of different microorganisms have been identified to accumulate PHA both aerobically and anaerobically.

The PHA stored inside the cells then needs to be extracted before being utilized in packaging, coating, agricultural, aquaculture, medical and pharmaceutical applications. As PHA is biodegradable and its physical and mechanical properties are similar to those of thermoplastics, such as polypropylene (PP), PHA has attracted much attention as a potential alternative for petrochemical plastics. Also, PHA can degrade in the natural environment by the enzymatic hydrolytic activities of depolymerase (PhaZ) secreted by microorganisms. The general structure of PHA is shown in Figure 1. A PHA molecule consists of the monomer units of (R)-hydroxy fatty acid. The monomeric units are connected to each other by an ester bond. Each monomeric unit has a side chain R group i.e. saturated alkyl group or unsaturated alkyl groups, substituted alkyl groups and branched alkyl groups [1]. To date, approximately 150 different types of monomers have been reported in the literature.



R	Nomenclature
CH_3	poly(3-hydroxybutyrate) (PHB)
C_2H_5	poly(3-hydroxyvalerate) (PHV)
C_3H_7	poly(3-hydroxyhexanoate) (PHHx)
C_5H_{11}	poly(3-hydroxyoctanoate) (PHO)

Figure 1 Formula of PHAs [2].

PHA has useful properties such as small pore size with high propensity to get recycled, high volume to surface ratio, biodegradability and biocompatibility, easy processing, good resistance to UV rays, insolubility in water and more. However, all of these PHAs have disadvantages, including high production cost, poor thermal mechanical properties, and unstable product quality associated with the current industrial biotechnology process [3].

Applications

The common applications of PHAs in the market include packaging, medical bioimplants, food-friendly films, agriculture, biofuel, drug delivery, tissue engineering and many more. Currently, more than 150 different monomeric units are known, making it possible to produce PHAs with various properties. PHAs are semi-crystalline polymers, and their thermal properties change depending on the monomer unit. These polymers consist of an amorphous and a crystalline part which acts as a physical crosslinking point. Their glass transition temperatures are between −35°C and 10°C and their melting points (Tm) are between 49°C and 177°C.

PHAs, could directly substitute PLA, another biopolymer, mainly produced from food-related resources such as corn, potato, etc., and from a sustainability point of view, the use of water and food resources to produce a biopolymer is controversial, in contrast PHAs are produced in the cell of bacteria. Another advantage is that PHAs are fully biodegradable compared with PLA, especially in drastic conditions, like when the PHA materials are released in the soil or in the aquatic environment [2]**.**

Additionally, the PHA family has a great potential to be applied in the 3D printing of biobased devices due to their biodegradability and non-toxicity which make them a great option for the fabrication of implants and bio-devices with direct contact with the human body. PHB and its PHBHV copolymers find use in implant applications due to being well tolerated by tissues when implanted under the skin of mice or in the muscles since no abscess formation or necrosis of the surrounding tissues has been observed.

In the aquaculture and health sectors, PHAs have been proposed as biocontrol agents, drug delivery systems, surgical sutures, cardiac valves, artificial skin, artificial organ reconstruction, and memory enhancers. It was reported that the addition of PHB to an aquaculture system resulted in a significant reduction of certain pathogens and improved the survival rate of Artemia nauplii against Vibrio campbellii LMG21363 infection. These synthons have properties that can serve as antimicrobial, antifungal, and antiviral agents, vitamins, flavors, and pheromones.

In the agricultural sector, their usage ranges from protecting plants from adverse environmental conditions, such as shading nets, clips, geotextiles, wires, and pheromone dispensers, as well as for the fabrication of low-cost bags for growing and transporting seedlings and mulching.

Biodegradability

One of the most important properties of PHAs is their complete biodegradability within three to nine months. A large number of aerobic and anaerobic bacteria and fungi are able to degrade PHAs thanks to their PHA depolymerizes. PHA is hydrolyzed into water soluble oligomers and monomers, which are utilized by the microorganisms as nutrients, and eventually degrade into carbon dioxide, methane and water as a byproduct of metabolism.

The degradation process is affected by the monomeric components, side chain length, and crystallinity. It has been reported that PHA films degrade faster than pellets and among other factors, higher temperature and humidity facilitate PHA degradation. In aerobic conditions, the end products of degradation are CO_2 and water. Under anaerobic conditions, methane is also produced. PHAs degrade into non-toxic oligomers being suitable candidates for in vivo use in medical applications.

Experiments carried out on biodegradability of PHA and PHB synthetized from peanut oil, demonstrated that the estimated values for maximal production of CO_2 (CO_2 max) were similar for both polymers. These CO_2 maximum values confirmed the complete mineralization of both polymers, making them suitable for green packaging applications [4].

Industrialization of PHAs

Even though there are several environmental advantages of PHA, their industrial production is limited because of the high cost of production (around 3–4 times higher than polypropylene or petroleum-based plastics). One of the fundamental reasons for this is the use of pure substrates like glucose, fructose, propionic acid, etc in the production process. Numerous studies have been performed to reduce the PHA production cost by employing waste substrates such as glycerol from biodiesel industries, lignocellulosic wastes, waste-cooking oil, agro-food wastes, and cheese whey from dairy industries. Using wastes and industrial byproduct streams as raw materials for PHA production can decrease the overall production cost by around 40–50% and can contribute to a circular economy scheme. Some examples of viable carbon substrates for PHA production include waste streams from food, dairy, sugar and biodiesel industries such as waste lipids, whey, molasses, lignin-rich residues and crude glycerol. The major influx in this industry is expected to come from waste from cooking oils primarily of plant origin (such as soybean oil, jatropha oil, palm oil, crude palm oil, palm kernel oil, and sludge palm oil). These oils are very inexpensive and usually have higher yields compared to other substrates as shown in Table 1.

Table 1 Examples of PHA production using lipid rich wastes by wild and recombinant strains, adapted and edited from [5].

Substrate		PHA Yield (g/L)
Plant Oil	Rubber Seed Oil	15.4
Plant Oil	Calophyllum Inophyllum Oil + Urea	10.6
Plant Oil	Coconut Oil	1.57
Plant Oil	Date Seed Oil	6.15
Plant Oil	Olive Oil Distillate	1.8
Plant Oil	Olive Mill Wastewater	2.8
Plant Oil	Canola Oil	3.2
Plant Oil	Date Oil	4.2
Plant Oil	Crude Palm Kernel Oil	6
Plant Oil	Crude Palm Kernel Oil	6.1
Plant Oil	Palm Oil	5
Plant Oil	Jatropha Oil	5
Waste Oil, Frying Oil, Oil Seed Cake	Waste Cooking Oil	1.2
Waste Oil, Frying Oil, Oil Seed Cake	Waste Cooking Oil	7.2
Waste Oil, Frying Oil, Oil Seed Cake	Waste Palm Oil	1.6
Waste Oil, Frying Oil, Oil Seed Cake	Waste Rapeseed Oil	2
Waste Oil, Frying Oil, Oil Seed Cake	Crude Palm Kernel Oil	2.1
Waste Oil, Frying Oil, Oil Seed Cake	Palm Oil Mill Effluent	10.2
Waste Oil, Frying Oil, Oil Seed Cake	Waste Frying Oil and Animal Fat	2.4
Glycerol (Biodiesel Waste)	Crude Glycerol + Oil Cake	1.07
Glycerol (Biodiesel Waste)	Crude Glycerol + Oil Cake	1.89
Glycerol (Biodiesel Waste)	Crude Glycerol	2.09
Glycerol (Biodiesel Waste)	Tallow	1.66
Glycerol (Biodiesel Waste)	Fish Oil Mixed with Glycerol	5.8
Glycerol (Biodiesel Waste)	Udder	35.6

Another of the limitations in the industrial production of PHA is the need to maintain the optimal bacterial growth conditions. More than 300 bacterial species have been reported to produce PHA. Most widely used microbes include Ralstonia, Burkholderia, Halomonas, Alcaligenes and Pseudomonas sp. because of their ability to utilize various carbon sources and produce different types of PHA. Bacteria under normal environmental conditions generate energy through channelizing organic matter through the tricarboxylic acid cycle (TCA) cycle. However, under stress conditions arising out of excess organic matter and limitations of nutritional elements such as nitrogen, potassium, phosphorus, oxygen, and magnesium, bacteria switch from energy producing (TCA cycle) to energy conserving pathway. Here, bacteria metabolize excess organic matter and divert acetyl CoA, the key intermediate of the TCA cycle toward

PHA synthesis. In bacteria, the most produced PHA is PHB, a polymer composed of β-hydroxybutyrate [6] [7].

Following the polymer extraction, several processes are used like isolation and purification of PHA. These processes contribute significantly to the overall PHA production cost. The main factors contributing to high recovery costs are the intracellular accumulation of PHAs and small amount produced by each cell. For the selection of an appropriate PHA recovery method, various factors should be considered: (i) the fragility of the cell wall, (ii) the type of PHA and quantity produced (high intracellular PHA concentration can increase the fragility of the cell and facilitate their release), (iii) the required purity (e.g. medical applications require higher purity), (iv) the impact of the extraction method over the PHAs' final molecular mass, (v) the overall costs (including production and downstream processing), and (vi) the environmental impacts. The most studied methods for PHA recovery consist of the following: (a) digestion of the non-PHA cell mass, (b) solvent extraction, and (c) mechanical disruption. Additionally, independent of the extraction process chosen, a pre-treatment step (e.g. heat, mechanical pre-treatment or use of alkali compounds and oxidants) is applied to weaken the cell wall in order to facilitate the extraction of the polymer and to achieve a higher recovery [8] [9].

Challenges and Future Research

PHAs have the potential to be very important biopolymers for the future and could replace other petroleum based and bio-based polymers that don't meet the ecological requirements needed. In order to massify PHA production, intensive research efforts still are needed for the identification of cheap feed materials and adequate microorganisms to withstand high variability in the waste stream, production of copolymers, and process engineering, leading to fully integrated and continuous production units.

There is also a novel promising alternative to be explored: new generation industrial biotechnology (NGIB) which employs contamination-resistant extremophilic bacteria to allow long-lasting, open and continuous, energy-saving bioprocessing, which uses low-cost substrates and less freshwater under artificial intelligence (AI) control.

References

[1] V. Sharma and et al., "Polyhydroxyalkanoate (PHA): Properties and Modifications," *Polymer,* vol. 212, 2020.

[2] M. Mehrpouya and et al., "Additive manufacturing of polyhydroxyalkanoates (PHAs) biopolymers: Materials, printing techniques, and applications," *Materials Science and Engineering C,* vol. 127, no. 112216, 2021.

[3] D. Tan, Y. Wang and et al., "Grand Challenges for Industrializing Polyhydroxyalkanoates (PHAs)," *Trends in Biotechnology,* vol. 39, no. 9, pp. 953-963, 2021.

[4] A. Pérez-Arauz and et al., "Production and characterization of biodegradable films of a novel polyhydroxyalkanoate (PHA) synthesized from peanut oil," *Food Packaging and Shelf Life,* vol. 20, no. 100297, 2019.

[5] R. G. Saratale and et al., "A comprehensive overview and recent advances on polyhydroxyalkanoates," *Bioresource Technology* , vol. 325, no. 124685, 2021.

[6] V. C. Kalia and et al., "Polyhydroxyalkanoates: Trends and advances toward biotechnological applications," *Bioresource Technology,* vol. 326, no. 124737 , 2021.

[7] M. Li and et al., "Recent advances in polyhydroxyalkanoate production: Feedstocks, strains and process developments," *International Journal of Biological Macromolecules,* vol. 156, pp. 691-703, 2020.

[8] L. de Donno Novelli and et al., "Polyhydroxyalkanoate (PHA) production via resource recovery from industrial waste streams: A review of techniques and perspectives," *Bioresource Technology,* vol. 331, no. 124985, 2021.

[9] B. Yadav and et al., "Concomitant production of value-added products with polyhydroxyalkanoate (PHA) synthesis: A review," *Bioresource Technology,* vol. 337, no. 125419 , 2021.

[10] "Potential Applications of Polyhydroxyalkanoates as a Biomaterial for the Aging Population," *Polymer Degradation and Stability journal,* vol. 181, no. 109371 , 2020.