How are polyhydroxyalkanoates (PHAs) produced?

By:
Rowyn Rose, Copywriter
Yuliana Mihaylova, CEO & Co-Founder, OliveBio, Los Angeles, CA, USA

Polyhydroxyalkanoates (PHAs) are aliphatic polyesters that can be synthesized by bacteria, archaebacteria, cyanobacteria, algae, fungi, and other microorganisms [1, 2]. This blog will dive into some of the main methods of PHA production, their benefits and constraints, and some of the proposed solutions that could lead to more sustainable and cost-effective production of PHAs.

Microbial fermentation for PHA production

Microbial fermentation involves a process whereby microorganisms, such as bacteria, archaebacteria, and fungi, break down large organic molecules (e.g., carbohydrates) into simpler ones [3]. Polyhydroxyalkanoates (PHAs) are synthesized via fermentation of organic compounds (e.g., glucose) and other carbon substrates inside a microorganism, which accumulate PHAs intracellularly as carbon and energy storage materials [2, 4, 5].

Different microorganisms and cultivation conditions can yield PHA homo- or copolyesters of 3-, 4-, 5-, and 6-hydroxyalkanoic acids. Poly(3-hydroxybutyrate) [P(3HB)] polymers, a well-researched subclass of PHAs, were the first to be discovered and commercialized [6]. The first phb gene was isolated from Zoogloea ramigera, which is an aerobic bacterium used to engineer biopolymers that produces both P(3HB) and extracellular polysaccharide. Since this discovery, many other genes that encode enzymes from PHA biosynthetic pathways have been cloned from different microorganisms [7].

There are also over 90 currently known genera of both gram-positive and gram-negative bacteria that can synthesize PHAs in both aerobic and anaerobic conditions [8]. There are over 300 bacteria, archaebacteria, and other microbial species known to date that have the capability to produce PHAs [9].

Bacteria can be categorized into two main groups when it comes to PHA production:

1. Bacteria that accumulate PHAs when nutrients (e.g., nitrogen, phosphorus, oxygen) are limited, but this accumulation does not occur during the growth phase in the cultivation medium [8, 10].

2. Bacteria that have no nutrient limitation requirements yet synthesize and accumulate PHAs during the growth phase in the cultivation medium [8, 10].

Carbon sources

Key to PHA synthesis in bacteria is the carbon source, as it affects the composition of PHAs produced by different bacterial strains [8]. These sources are broadly categorized as starch-based, sugar-based, cellulosic, hemi-cellulosic, whey-based, and oil- and glycerol-based media.

As shown in Figure 1, these can be further simplified into three general substrate categories: carbohydrates, hydrocarbons, and triacylglycerols [11].

Bacteria convert the substrate/carbon source into PHA biopolymers through a series of enzymatic reactions. To trigger PHA production, the nutrient supply must be carefully managed. When bacterial growth is limited by the depletion of nutrients, such as nitrogen or phosphorus, bacteria are naturally prompted to store carbon and energy [12, 13]. Nitrogen limitation is a common strategy to redirect many microorganisms' metabolic pathways toward PHA synthesis, while for bacteria such as Azotobacter spp., limiting oxygen is more effective for synthesis [12, 14].

Fermentation conditions

The physical properties and degradation rate of PHAs can be modified by changing the bacterial source and the fermentation conditions [10]. These fermentation processes for microbial production of PHAs take place in controlled environments, where factors such as temperature, pH, oxygen levels, and agitation are optimized for microbial growth and PHA accumulation. Three main bacterial cultivation strategies include [14, 15]:

1. Batch fermentation: this common method is known for its simplicity, flexibility, and low operation costs. The substrate and other requirements are added to the bioreactor at the beginning, allowed to react with one another, and then after the reaction the PHA material can be extracted. However, studies have shown that polymer composition is not constant over time (especially as compared to continuous/chemostat cultures) [16].

2. Fed-batch fermentation: also known as semi-batch fermentation, this favoured strategy is considered the most efficient for achieving high cell density cultivation, high yield, and high performance. During operation, this process involves adjusting culture broth feed rates by adding nutrients needed for cell growth or product formation in the culture vessel – either intermittently or continuously – to keep the concentration of limiting nutrients at an optimal level. Productivity is increased while overall fermentation time is decreased [17].

3. Continuous fermentation: also known as chemostat fermentation, this method involves continuously replacing culture broth with sterile medium. This steady-state process requires that the microbial culture be continuously fed nutrients at a fixed rate, while simultaneously being harvested to keep culture volume constant. Since physiological parameters of cultures in steady-state do not change over time (theoretically), growth conditions can be studied in a time-independent manner [16].

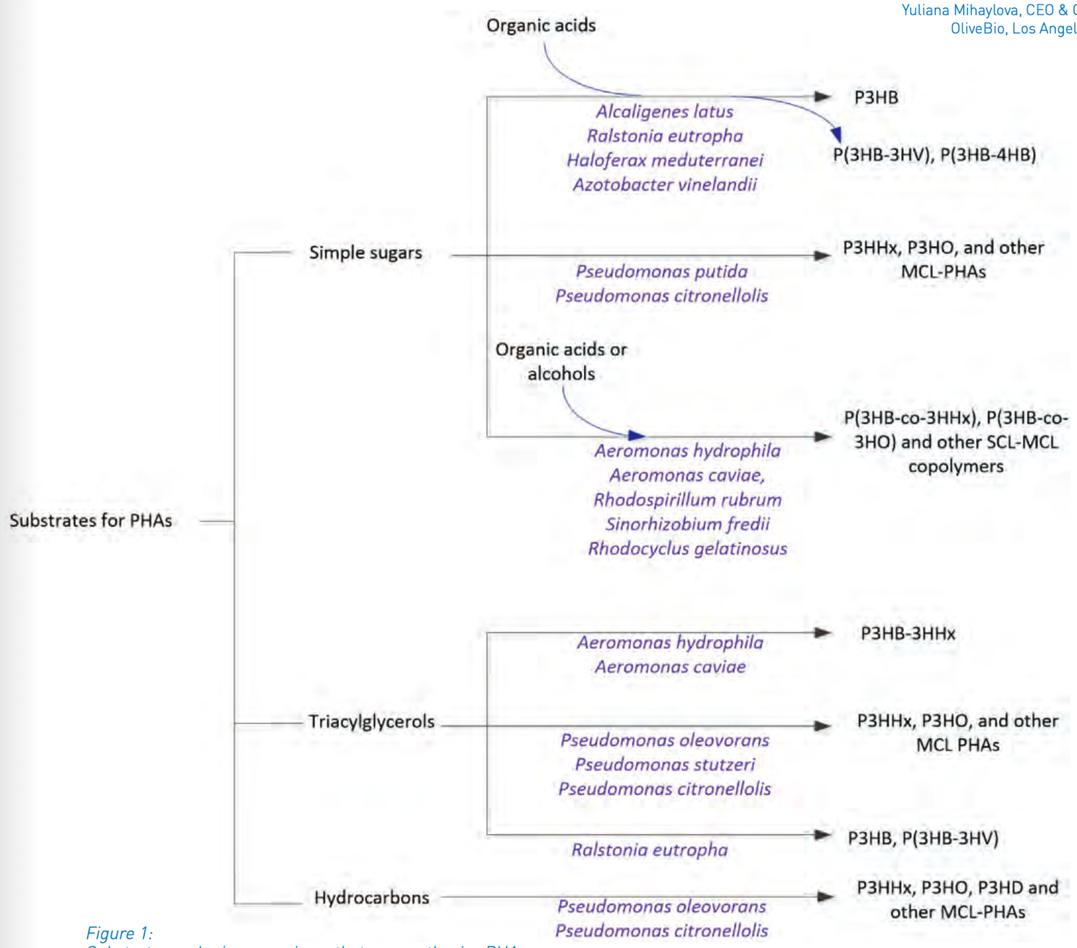

Figure 1:
Substrates and microorganisms that can synthesize PHAs.

Extraction of PHAs

After fermentation, PHA needs to be extracted from the bacterial cells and purified. Table 1 reflects the typical steps for downstream processing associated with bacterial synthesis of PHAs, along with examples of methods for each step [18]:

Biomass Separation	Pre-Treatment	PHA Recovery	PHA Separation	PHA Purification
■ Filtration ■ Centrifugation ■ Sedimentation	■ Heating or freezing ■ Adding salt ■ Adding chelating agents	■ Solvent extraction ■ Digestion methods ■ Mechanical disruption	■ Precipitation ■ Centrifugation ■ Sedimentation	■ Redissolution ■ Activated charcoal ■ Ozone

Table1:
Steps in PHA downstream processing and example methods for each step (adapted from del Oso et al., 2021) [18]

The below list provides more detail on some of these downstream processing methods, specifically with regard to extraction/recovery of PHAs [8]:

1. Solvent extraction: this is considered the most common method for PHA extraction. First, bacterial cells are ruptured through a pre-treatment process that exposes PHA granules. The granules are then solubilised in chloroform or other chlorinated solvents, and finally precipitated with ethanol or methanol. This method does not degrade the polymer, removes cell endotoxins, and creates a high purity PHA product. However, halogenated solvents like chloroform require a lot of energy input (which increases production costs) and are harmful to the environment [19].

2. Flotation method: this process first uses a solvent such as chloroform to extract PHAs from bacterial cells, with an added step of leaving this cell-chloroform mixture for a period of time so that flotation of cell debris can occur. This method can employ the use of environmentally friendly solvents, and that the process is known to reduce wastage of the extracted polymer [8].

3. Digestion method: as an alternative to solvent extraction, this method releases PHAs from bacterial cells via chemical or enzymatic digestion. Chemical digestion involves sodium hypochlorite or surfactants to recover PHAs from cells, while the enzymatic method involves heat treatment, enzymatic hydrolysis, and surfactant washing. However, chemical digestion can be problematic if toxic substances such as chloroform are used [8].

4. Supercritical fluid extraction: by raising the temperature and pressure of a substance beyond its critical point, one can create highly compressed fluids that combine the properties of both gases and liquids. Supercritical carbon dioxide, ammonia, and methanol extraction have been used to recover PHAs from bacterial cells. This method is effective at extracting endotoxins and other impurities that may remain after solvent extraction, which is very important for producing PHAs used in vivo for biomedical applications [8, 20].

5. Aqueous two-phase extraction (ATPE): as a non-solvent method, ATPE is considered an ecologically friendly approach. It uses water to isolate, purify, and recover PHAs from bacterial cells [8].

Though final PHA production costs depend on a variety of factors, downstream processing is said to be responsible for 50 % or more of the total cost of production [21, 22].

Constraints of microbial fermentation for PHA production

Microbial fermentation is currently a main industrial biotechnology method for producing polymers such as PHA. This method of production is associated with high energy consumption and maintenance costs for tasks such as sterilization, oxygen supply, and agitation—all of which contribute to high overall PHA production costs [23]. Microbial fermentation methods also face obstacles [19, 24, 25] related to:

- Frequent microbial contamination (and high capital investment for facilities/equipment needed to mitigate contamination),
- High energy and water resource needs,
- Difficulty with recycling culture broth,
- Complex and costly product separation and purification (e.g., downstream processing),
- Low substrate-to-product conversion efficiency, and
- Lack of platform microbial strains to develop multiple products in shorter time periods.

Other considerations regarding microbial fermentation for PHA production include:

- Fermentation/cultivation strategies: each of the three main fermentation strategies has its benefits, as well as drawbacks
  - Batch fermentation has the lowest productivity out of the group since the accumulated PHA begins to deteriorate after the carbon source is fully expended, resulting in an overall decrease in PHA material.
  - The overall PHA production of fed-batch cultivation is considered low when nitrogen is the limited nutrient; combining batch and fed-batch processes has become a common fermentation strategy for higher productivity.
  - Though chemostat/continuous fermentation is known for high controllability, the continuous feeding process for PHA build-up, yield, and productivity is also associated with a higher chance for contamination [15].
- Bacterial strains: efficiency of PHA production varies among different strains. The development of genetically engineered strains with higher PHA accumulation rates and broader substrate utilization is one of the methods being explored to increase efficiency and yields [26].
- Scaling: scaling up PHA production from lab-scale to industrial-scale can be complex. In addition to high costs, challenges such as maintaining optimal conditions, preventing contamination, and achieving consistent PHA quality are among the list of hurdles.

- Sustainability of carbon sources: sustainability of carbon sources used in PHA production is critical. Researchers are exploring alternative feedstocks, such as lignocellulosic biomass and agricultural waste, to reduce the reliance on food-based substrates that require large amounts of land, water, and other resources to maintain (e.g., [15, 27]).

Despite these challenges, research and innovation hold the promise for efficient methods for PHA production via microbial fermentation. For example, microbial fermentation methods for PHA production began with the use of pure bacterial cultures, with a pure concentrated substrate (e.g., glucose). However, PHAs can also be produced in open mixed-culture systems using diverse microbial communities and a broader range of substrates, including activated sludge from wastewater treatment plants [19]. These systems allow for the use of a wider variety of cheaper carbon sources and continue to pique interest due to their ability to produce higher PHA yields and lower overall production costs [26, 28, 29]. ■

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References:

[1] https://doi.org/10.1016/S1369-703X(03)00027-5 Taguchi et al., 2003
[2] https://doi.org/10.1016/j.chemosphere.2022.133723 Behera et al., 2022
[3] https://doi.org/10.3390/fermentation6040106 Sharma et al., 2020
[4] https://doi.org/10.1155 %2F2013 %2F752821 Bhuwal et al., 2013
[5] https://doi.org/10.1007/s11270-022-06029-2 Acharjee et al., 2023
[6] https://doi.org/10.1016/B978-0-444-63662-1.00029-4 Tan, 2017
[7] https://doi.org/10.1128/mmbr.63.1.21-53.1999 Madison & Huisman, 1999
[8] https://doi.org/10.1016/j.ibiod.2017.10.001 Raza et al., 2018
[9] https://doi.org/10.1016/j.biortech.2021.125419 Yadav et al. (2021)
[10] https://doi.org/10.1002/jctb.2392 Akaraonye et al., 2010
[11] https://doi.org/10.3390/ijms17071157 Jiang et al., 2016
[12] https://doi.org/10.1111/j.1365-2672.2007.03335.x Verlinden et al., 2007
[13] https://doi.org/10.1023/A:1025047410699 Shang et al., 2003
[14] https://doi.org/10.3303/CET1332156 Amache et al., 2013
[15] https://doi.org/10.3390/life11080807 Vigneswari et al., 2021
[16] https://doi.org/10.1016/bs.mie.2019.08.018 Amstutz et al., 2019
[17] https://doi.org/10.3390 %2Fmicroorganisms8020151 Poontawee & Limtong, 2020
[18] https://doi.org/10.1016/j.cej.2020.127687 del Oso et al., 2021
[19] https://doi.org/10.3390 %2Fpolym14112155 Rodrigues et al., 2022
[20] https://olivebio.com/what-are-the-different-applications-for-polyhydroxyalkanoates-phas
[21] https://doi.org/10.1016/j.biortech.2021.124685 Saratale et al., 2021
[22] https://doi.org/10.1080 %2F21655979.2021.1935524 Vu et al., 2021
[23] https://doi.org/10.3390/polym15102333 Dong et al., 2023
[24] https://doi.org/10.1016/j.copbio.2017.11.016 Chen & Jiang, 2018
[25] https://doi.org/10.1016/j.aiepr.2019.11.001 Chen et al., 2020
[26] https://doi.org/10.3390/bioengineering4020055 Kourmentza et al., 2017
[27] https://doi.org/10.1021/bm049478b Koller et al., 2005
[28] https://doi.org/10.1016/j.biotechadv.2003.09.003 Salehizadeh & Van Loosdrecht, 2004
[29] https://doi.org/10.1016/j.biortech.2006.10.046 Gurieff and Lant 2007