Current Applied Science and Technology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2025, Vol. 25 (No. 6), e0261159

Review article

Metabolic Process and Types of Carbon Source leads to Desired Polyhydroxyalkanoate Properties

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Received: 4 December 2023, Revised: 27 September 2024, Accepted: 17 November 2024, Published: 22 April 2025

Abstract

Polyhydroxyalkanoates (PHAs) have recently been focused due to increasing public awareness of environmental issues. A lot of efforts have been made to understand the mechanisms of biosynthesis of PHA homo or copolymers. PHAs of varying monomer composition have been produced using a number of carbon sources and organisms. Carbon sources played a major role in determination of homo/copolymers. The metabolic processes leading to PHA biosynthesis were analyzed, aiming to differentiate their effect on production of homo/copolymers. Moreover, the roles of carbon source and/or organism behind physical and chemical properties of PHAs were evaluated. One key strategy in the production of a novel copolymer PHA is to engineer a strain with desired genes that can be used for desired copolymer production. It engineered strain could be able to design and optimize the metabolic process to produce more diverse polymers. Development of more efficient regulatory factor to produce PHAs of selective monomer composition and properties was focused in this review.

Keywords: polyhydroxyalkanoates; copolymer; metabolic process; carbon source; properties

1. Introduction

The growing need for renewable-resource-based and environmentally friendly products has stimulated extensive research in a range of fields. Moreover, due to limited reserve of petroleum, it has become crucial to find new alternative and sustainable sources of materials that can be used by microorganisms to produce materials such as polymers and plastics that can act as sustainable alternatives to petroleum-based plastics.

Conventional plastics, mainly manufactured from fossil fuel, are non-biodegradable and therefore strongly resistant to microbial attack. These plastics accumulate in ecosystems, making a huge negative impact on our environment. Moreover, every year a number of new plastic products with favorable properties and strong durability enter the global market, and at the end of their life are discharged into the environment. The durable characteristics of plastics can contribute to the accumulation of plastic wastes

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https://doi.org/10.55003/cast.2025.261159

both on land and in aquatic systems. Microplastics derived from a number of plastic products have exacerbated the plastic problem, which is now one of the most serious global crises; threatening ecosystems and human health (Kim & Kim, 2018; Herrera et al., 2019; Koller, 2019).

Globally, annual plastic production and consumption have increased exponentially. Plastic-based products with desirable material properties such as lightness, clarity, and durability can be produced at a very low price. Nowadays, due to a number of advantages and versatile applications, it has become hard to reduce the consumption of them. The possibility of replacing petroleum-based plastics with bio-based and biodegradable materials has given hope to reduce the negative consequences of conventional plastics. Bioplastics are biodegradable, produced from renewable resources through microbial cultures, and generate non-toxic waste at the end of use. These materials can be regarded as environmentally friendly alternatives to conventional plastics since the release of excess carbon as carbon-dioxide, the main factor contributing to global warming, can be minimized and reduced the plastic waste problem. A number of researchers have studied polyhydroxyalkanoates (PHAs) and in particular polylactic acid (PLA) to improve their properties as well as their potential application in a range of products from the industrial through to the household and medical.

Amid different types of bioplastics, PHAs are well accepted for most favorable physical properties to thermoplastics and elastomers and thus have great potential to substitute for conventional plastics (Palmeiro-Sánchez et al., 2022). PHAs are produced through a complete biosynthetic way from stored renewable or waste organic materials that are the intracellular storage materials for carbon and energy sources of a number of organisms. The biocompatible and biodegradable nature and flexible mechanical and thermal properties of PHAs have made them attract interest (Sharma et al., 2021; Palmeiro-Sánchez et al., 2022).

PHAs are produced mainly from renewable resources such as sugars, molasses, glycerol, methanol, organic waste, organic acids and even carbon dioxide (Kucera et al., 2018; Wang et al., 2020; Wen et al., 2020; Kiselev et al., 2022). PHAs have several advantages such as reducing the threat to fossil fuel reserves and environmental pollution associated with the disposal of non-biodegradable plastics. This production and use of PHAs is also considered as green technology. It involves no emission of CO_2 into atmosphere as it is produced from renewable resources and is part of natural carbon cycle (Koller, 2019).

There are many reasons behind the increasing interest in the bioproduction of PHAs. Firstly, they are biodegradable and have many specific properties comparable to conventional plastics. Secondly, the production of PHAs through chemical synthetic methods is very difficult and is not economically feasible even though the structure of PHAs is not too complex. Biosynthesis of PHAs using microorganisms is relatively cheap compared to chemical synthesis of PHAs (although the costs are still higher than the costs of synthetic polymer synthesis). They are generally produced by fermentation in both heterotrophic and/or autotrophic production and in principle from renewable organic resources or CO_2.

Several intensive studies of PHAs production and application have been conducted. Most of them were concerned with finding cheaper carbon sources to reduce costs or applying genetic engineering to organisms with the aim of improving the product quality and productivity. Most of these works were conducted with heterotrophic cultures using organic materials as carbon and energy source and effectively produced PHAs with high concentration and productivity (Kim et al., 1994; Steinbüchel, 2001; Mozumder et al., 2014).

However, attempts at producing PHAs from CO_2 by hydrogen oxidizing bacteria are still rear because of some difficulties of autotrophic cultures, the use of CO_2 as carbon

source, and the high cost of hydrogen as energy source (Garcia-Gonzalez et al., 2015; Ghysels et al., 2018). PHAs are polyester produced by a wide range of organisms containing numerous hydroxyalkanoic acids. Till now a number of basic and applied research have been conducted to gather knowledge on biochemical and molecular levels and to follow the enzymatic interactions on the biosynthesis of PHAs. Detailed physiological studies, the determination of metabolic pathways of natural and engineered organisms, the interaction of enzymes within the metabolic reactions revealed new perspectives in the production of new and diverse homo/copolymer of PHAs with desired properties (Choi et al., 2020; Mitra et al., 2022), and opened new windows for technological advancement.

Complete knowledge of microbial metabolic behavior is very important when designing a novel PHA polymer with the desired properties. Accumulation of PHAs in bacterial cells occurs with access to carbon sources and is enhanced by depletion of one of the nutrients that are essential for growth. Generally, PHAs accumulate in cytoplasm of organisms as cytoplasmic inclusions such as PHA granules (Steinbuchel et al., 1995). The availability of spaces in cytoplasm determines PHA production capacity as well as PHA content (Steinbüchel, 2001). Different types of PHAs (homo or co) were produced from deviating carbon source or interfering in the microbial metabolism that produced divert or novel intermediates as well as derivation of hydroxyacyl-CoA thioesters (Madison & Huisman, 1999). The thioesters were then polymerized by PHA synthases (Rehm & Steinbüchel, 1999; Steinbüchel & Hein, 2001) by bonding with the surface of PHAs granules (Pieper-Fürst et al., 1994; Wieczorek et al., 1995). Most of these PHAs were only obtained from structurally related CoA-thioesters that were produced from related carbon sources. Therefore, few PHAs were able to be produced from simple carbon sources that were available as renewable resource such as agricultural products, organic wastes from industry and CO_2 (Steinbüchel, 2001).

There is still ongoing research to find novel copolymer PHAs that feature new or novel constituents, contain different combinations of known constituents, or new orders of constituents (block copolymer or random copolymer). The produced PHA monomers were mainly governed by the type of carbon source. The biosynthesis of PHA monomers was controlled by PHA synthases that were very specific to carbon source and produced following a specific metabolic pathway (Koller & Mukherjee, 2022).

There are three steps in the metabolism of carbon source to biosynthesis of PHA monomers; (i) Specific transportation or passive transportation that transport the carbon source into the bacterial cell, (ii) production of specific thioester as an intermediate from the carbon source and (iii) conversion of thioester-CoA to PHA monomers (Pötter & Steinbüchel, 2006; Chia et al., 2010). Moreover, novel PHAs with desired constituents and properties were possible to be produced either by using a mixture of carbon source (Chia et al., 2010) or applying a novel enzyme for PHA synthesis (Wróbel et al., 2004; Mochizuki, 2005) or by using genetically modified organisms (Park, et al., 2012b; Park et al., 2013). Such studies showed that novel copolymer could be satisfactorily produced with improved and desired properties that raised application potentiality.

In this review, the metabolic processes based on carbon sources that led to homo or copolymer PHA production were analyzed, with the aim to differentiate the factors that affected the production of homo or copolymers. Moreover, the roles of carbon sources and/or organisms behind the determination of physical and chemical properties as well as the type of PHAs were evaluated. The properties of the synthesized PHAs based on their production system with focus on organisms and carbon sources were summarized.

2. Chemical Structure of PHAs

The monomer of a PHA is a hydroxyalkanoic acid, and is in a form of polyester when polymerized. Figure 1 shows that the most common structure of PHA polyester is composed of 3-hydroxyalkanoic acid. The pendant group (R) varies from methyl (C1) to tridecyl (C13) (Madison & Huisman, 1999). If the R group is the methyl ($-CH_3$) group, the PHA is poly(3-hydroxybutyrate), or poly(3HB) for short, but if it is the ethyl ($-CH_2CH_3$) group, the polymer is poly(3-hydroxyvalerate), or poly(3HV). If the R group is the prophyl ($-CH_2CH_2CH_3$) group, the polymer is poly(3-hydroxyhexanoate), or poly(3HHx). Structurally related carbon sources lead to the production of similar hydroxyalkanoate monomers as well as homopolymer, but structurally unrelated carbon sources generate different types of monomers that build copolymers. Fatty acids monomers with saturated or unsaturated pendant groups and hydroxyl groups at positions 4, 5 or 6 show similar structural behavior and hence lead to the production of homopolymers. In the polymerization reaction, an ester bond is formed through the binding of carboxylate group of one monomer and hydroxyl group of other monomer that was catalyzed by host's PHA synthase (Verlinden et al., 2007). There was possible to make variation in composition of side chain and change the length of produced PHAs suitable for potential application (Donaruma, 1991).

When a mixed carbon source is used, microorganisms convert it into PHA copolymers such as poly(3HB-*co*-3HV) and poly(3HB-*co*-4HB). The microstructure of block or random PHA copolymers could be controlled using innovative technique of feeding of the carbon source. In production of poly(3HB-*co*-3HV) using *Cupriavidus necator*, 3HB monomer units were produced from fructose and 3HV monomers were produced from pentanoic acid. Random copolymers were produced through the pulse feeding of pentanoic acid. For the synthesis of a block copolymer with the desired 3HV composition, accurate control of pentanoic acid co-feeding time to the bioreactor was very important (Pederson et al., 2006).

PHAs were classified in two distinct groups based on the number of carbon atoms in monomers: (i) 3-5 carbon atoms in the monomer were known as short chain length PHAs (scl-PHAs) and monomers with 6-14 carbon atoms were medium chain length PHAs (mcl-PHAs). Poly(3HB) and poly(3HB-*co*-3HV) are examples of scl-PHAs and poly(3HHx-*co*-3HO) is a member of the mcl-PHAs. Microorganisms like *Cuprivadus necator*, *Alcaligenes latus*, and *Bacillus thuringiensis* produced scl-PHAs (Steinbüchel & Schlegel, 1991; Ponnusamy et al., 2019) whereas the medium chain length polymers were produced by *Pseudomonas putida* and *Pseudomonas mendocina* (Borrero-de Acuña et al., 2014; Anis et al., 2018).

3. Metabolic Pathway of PHA Production

Metabolism means the biochemical reactions and processes accountable for chemical transformations within the cells of living organisms including the processes of formation, breakdown and inter-conversion of carbon sources. There are usually two types of metabolism; catabolism, which involves the breakdown of organic matter and the harvest of energy through respiration, and anabolism, which involves the use of energy to produce the components necessary for a cell's body construction or product formation. The complete reaction cycles involved in metabolism are known as metabolic pathways. A wide range of organisms show similar metabolic processes and intermediate components (Pace, 2001). In the metabolic process, the role of enzymes is very crucial. They allow organisms to drive desire reactions that would not occur by themselves, reduced energy activation in such a way that the reactions occur spontaneously, and later the energy was

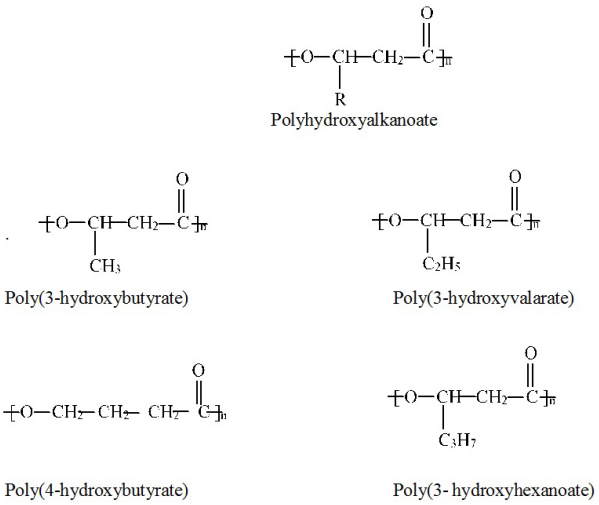

Figure 1. Typical structures of polyhydroxyalkanoates

released. Enzymes allow reactions to occur more rapidly and can regulate the metabolic process by changing the process environment through transporting the signals from the cells. The secretion of enzymes that determined the metabolic pathway mainly depended on the carbon source that was used for cell construction and PHA production.

3.1 Simple carbohydrates

Most PHAs producing organisms do not have the ability to metabolize complex carbohydrates but they can easily oxidize simple carbohydrates. Different organisms follow their own specific enzymatic oxidation pathway. The simplest and most important carbohydrates that have been used for PHA production were glucose and fructose, which are metabolized by nearly all known organisms. The well-known metabolic pathway of glucose is glycolysis, which involves the conversion of glucose into pyruvate and then into Acetyl-CoA. This reaction also releases sufficient amount of free energy which can produce high energy compounds like NADH (reduced nicotinamide adenine dinucleotide) and ATP (adenosine triphosphate). Glycolysis consists of a number of enzyme-catalyzed reactions with a number of intermediate products. The intermediates can also provide entry points of carbon sources other than glycolysis of glucose (Figure 2).

Enter-Doudoroff (ED) metabolic pathway is the most common glycolysis process. In this glycolysis pathway, glucose is first converted to glucose-6-phosthate through phosphorylation of glucose by hexokinase and is then isomerized to 2-keto-3-deoxy-6-phosphogluconate. In case of fructose, fructose-6-phosphate is directly produced by phosphorylation and enters the glycolysis pathway (Figure 2). Genome analysis of *Ralstonia eutropha* (presently known as *C. necator*), the most common organism able to produce PHAs with a higher capacity, was identified that the degradation of fructose in cell chromosomes was conducted by three clusters of genes.

Cluster 1 (*frcRACBK*, *pgi2*, and *zwf2*) were significant in the transformation of fructose to 6-phosphogluconolactone, cluster 2 (*glk*, *zwf3*, *pgl*, and *edd2*) were involved in sugar phosphorylation and Enter-Doudoroff (ED) pathway and cluster 3 (putative 2-amino-2-deoxy-D-hydrolase, *kdgK* and *eda*) took part in hydrolysis, degradation of glucosaminate and also took part in ED metabolism (Shimizu et al., 2013). Moreover, it was assumed that

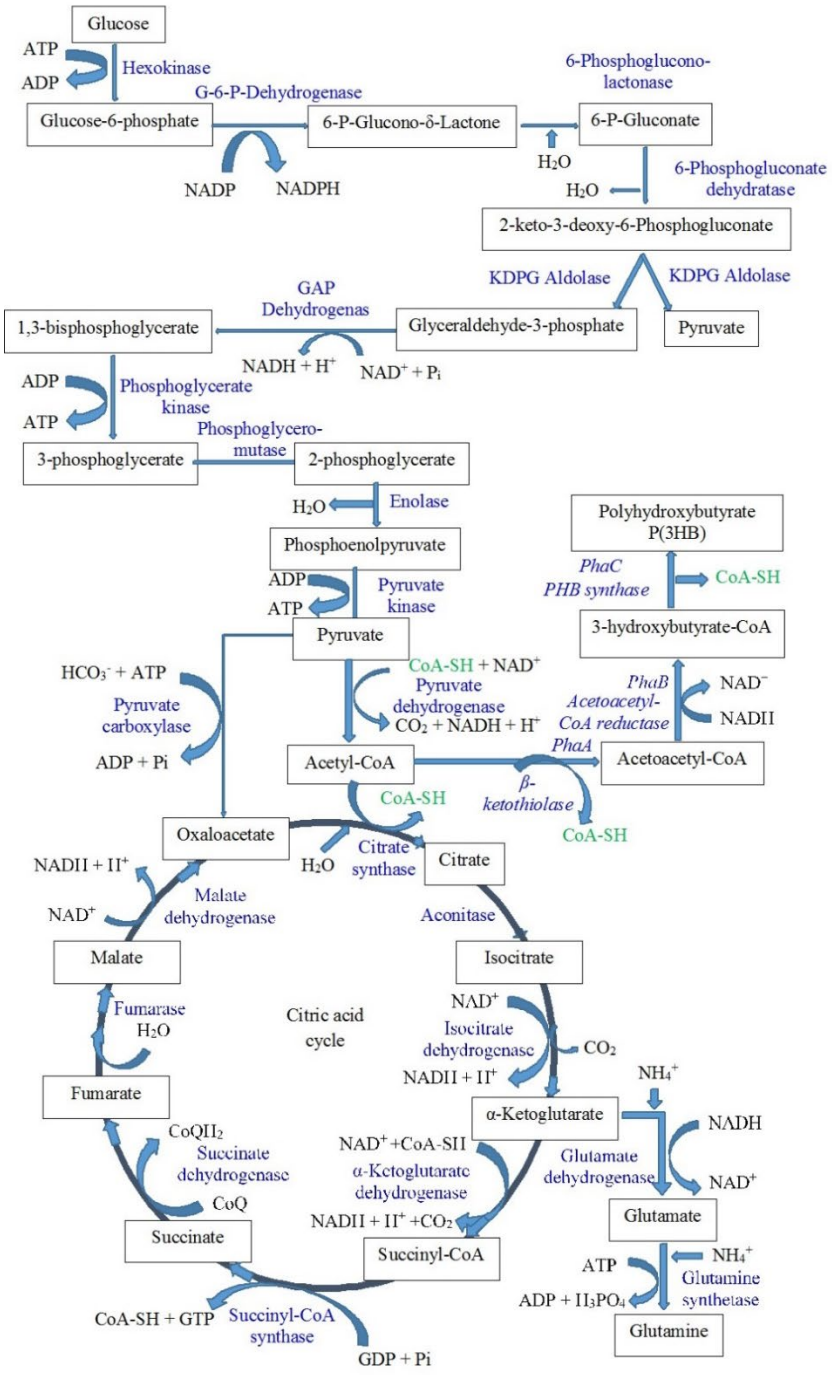

Figure 2. Metabolic network of PHA producing bacteria through Enter-Doudoroff (ED) pathway for growth and PHB production over simple carbohydrate (adopted from Ackermann & Babel, 1997; Kayser et al., 2005; Shimizu et al., 2013)

Embden-Meyerhof (EM) pathway was active in the production of pyruvate from glyceraldehyde-3-phosphate that was produced by ED pathway. The EM pathway was also shown to be involved in the gluconeogenesis of non-sugar hydrocarbons such as glycerol. Glycerol was first phosphorylated, and this was followed by dehydrogenation to dihydroxyacetone-phosphate, then was converted to glyceraldehyde-3-phosphate and entered into the glycolysis pathway to produce pyruvate (Chen et al., 2019a). Mainly, three genes that were incorporated into the pyruvate dehydrogenase complex; *pdhA1*, *pdhB*, and *pdhL*, were highly induced in the growth phase. In particular, *pdhL* exhibited 18.5 times higher activities compared to PHA production phase. The produced pyruvate either follows TCA-cycle for microbial growth or glyoxylate bypass for PHA production (Figure 2). The specific genes determined the carbon flux either to the TCA cycle or the glyoxylate bypass. Under stress conditions, the genetic activities of *iclA* and *iclB* were highly induced that lead to PHA production phase. The activity of *iclB* was 33 times higher compared to the growth phase ensuring the glyoxylate bypass became the predominant one. The decrease of carbon flux into central metabolism was detected by the lower intercellular concentrations of many sugar-phosphates in the PHA production stage (Shimizu et al., 2013). It was assumed that metabolic activities were decreased in PHA production stage but enough for maintaining the cell viability and conversion to PHA, without any growth taking place.

Bacterium like *C. necator* followed a three step process in the conversion of acetyl-CoA to poly(3HB) (Steinbüchel & Schlegel, 1991). The first conversion to acetoacetyl-CoA was catalyzed by 3-ketothiolase (*phaA*) and was then converted to (R)-3-hydroxybutyryl-CoA that was enhanced by enzyme pyridine nucleotide-dependent acetoacetyl-CoA reductase (*phaB*). Finally, poly(3HB) was produced from (R)-3-hydroxybutyryl-CoA by PHA synthase (*phaC*). Mainly, scl-PHAs such as poly(3HB) and poly(3HB-*co*-3HV) were produced through this biosynthesis pathway (Choi et al., 2020). The gene involved in glyoxylate bypass pathway to produce (R)-3 hydroxybutyryl-CoA from acetoacetyl-CoA was *phaB* and production of poly(3HB) was performed by the gene phaZ (Shimizu et al., 2013). It was also possible to produce 2-hydroxybutyrate (2HB) monomer as well as poly(3HB-*co*-2HB) using pure culture and pure hydrocarbon (such as glucose) through the citramalate pathway that produced 2-ketobutyrate from pyruvate and then to 2-hydroxybutyryl-CoA (Atsumi & Liao, 2008). In this pathway (Figure 3), the *cimA* gene was responsible for the production of (R)-citramalate, the *leuB* genes for 2-ketobutyrate, the *panE* for 2-hydroxybutyrate and the *Pct540Cp* for 2-hydroxybutyryl-CoA (Park, et al., 2012b). It was possible to produce the 4-hydroxybutyrate (4HB) monomer or poly(3HB-*co*-4HB) polymer if the *sucD*, *4hbD* and *ortZ* genes were present. The *sucD* gene was responsible for the conversion of succinyl-CoA to succinic semi-aldehyde in the TAC cycle (Figures 2 and 3) and was converted to 4-hydroxybutyrate by *4hbD* and to 4-hydroxybutyryl-CoA by *ortZ* (Figure 3) (Park, et al., 2012a). A recombinant *Ralstonia eutropha* was created with the introduction of a new gene propionate-CoA transferase (*pct*) from *Megasphaera elsdenii*. It produced a copolymer poly(3-HB-*co*-3-HV-*co*-2,3-di-HB) using glycolate. Interruption of *phaA* (3-ketothiolase) in the *phaCAB* operon increased 2,3-di-HB composition by up to 3 mol% in of PHA copolymers (Insomphun et al., 2017). It was possible to produce PHA copolymers that contained several monomers from single carbon sources and single (genetically modified) organisms. But for doing so, it was important to genetically modify the organisms in such a way that it would increase the activities of specific genes to follow the different pathway at the same time. Moreover, one recent research showed that the citrate synthase genes redirected metabolic flux from central metabolic pathways to the PHBV synthesis pathway and enhanced the productivity of the PHBV copolymers (Lin et al., 2021).

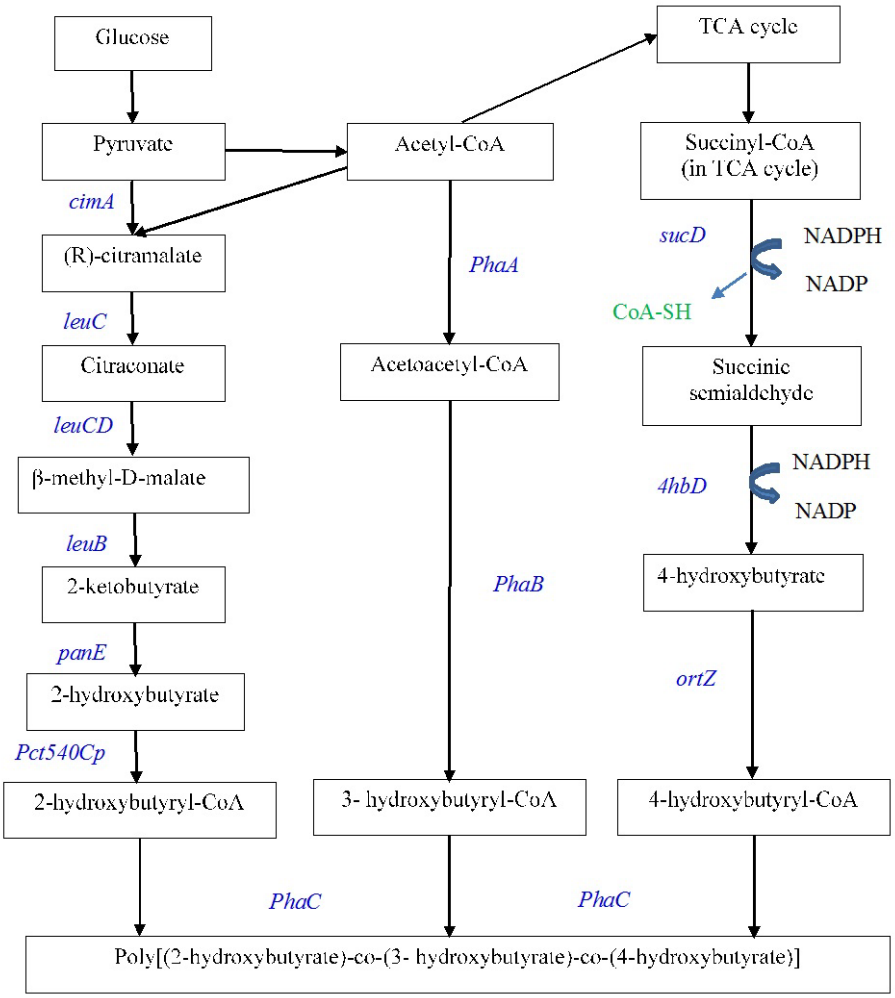

Figure 3. Metabolic pathways for the production of PHAs containing 2HB monomer through citramalate pathway (Park, et al., 2012b) and 4HB monomer from TCA cycle (Park, et al., 2012a)

3.2 Non-sugar carbon source

A number of studies were conducted to produce PHAs using fatty acids (commonly used propionic acid, which is also known as propionate). In the metabolic process for PHA production, the fatty acid predominantly followed oxidation pathway. The oxidation took place in methyl group at either the α-position or the β-position in fatty acid. Through the α-oxidation pathway, propionyl-CoA was first produced and then converted to acetyl-CoA (Figure 4) (Silva et al., 2000). The metabolic pathways of fatty acids were discussed in a number of literatures (Hörster & Hoffmann, 2004; Park et al., 2013; Silva et al., 2000) and a complete pathway of PHA production via α-oxidation is summarized in Figure 4.

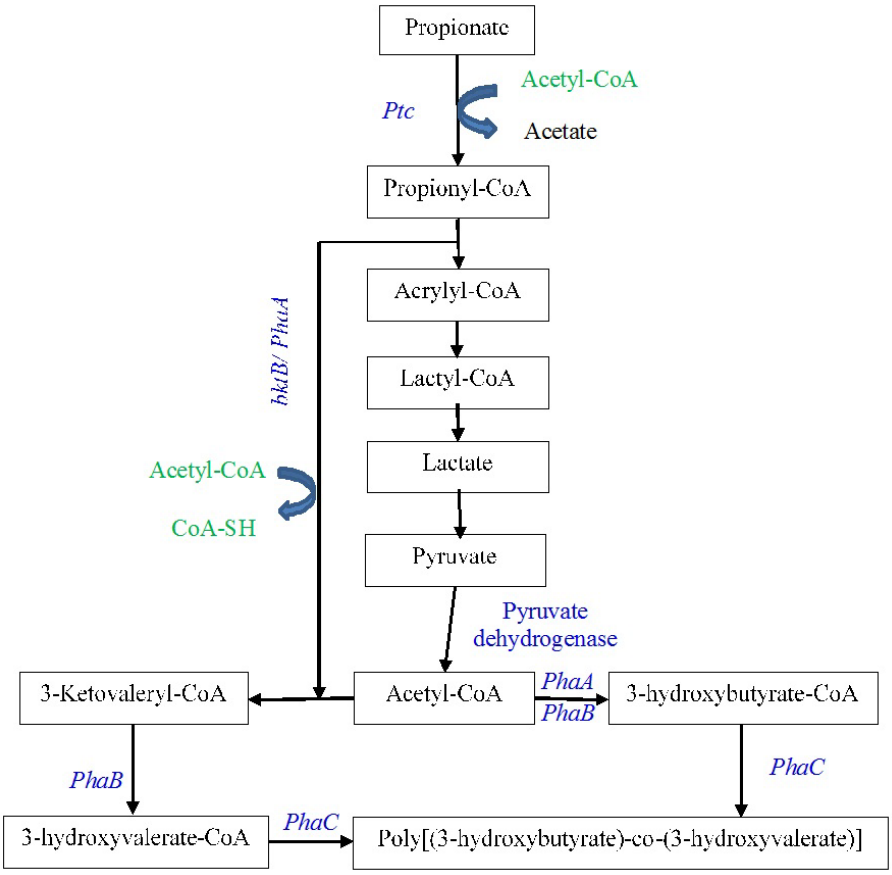

Figure 4. Metabolic network for the production on PHA copolymers from fatty acids via α-oxidation (Silva et al., 2000; Wang et al., 2014; Chen et al., 2018)

Most of the previous authors (Silva et al., 2000; Hörster & Hoffmann, 2004; Park et al., 2013) considered that the fatty acid contained only odd number of carbons ultimately responsible for α-oxidation. Shimizu et al. (2013) showed the complete metabolic pathway of fatty acids that followed β-oxidation, as shown in Figure 5. The metabolic pathway for production of acetyl-CoA from propionyl-CoA and the production of 3-Ketovaleryl-CoA were determined by oxidation process; in α-oxidation, propionyl-CoA was first converted to pyruvate and then to acetyl-CoA (Figure 4) (Pernicova et al., 2019). But under β-oxidation, propionyl-CoA was first converted to 3-ketovaleryl-CoA and then to acetyl-CoA (Figure 5) (Shimizu et al., 2013). During α-oxidation, 3-ketovaleryl-CoA was produced from the combination of propionyl-CoA and acetyl-CoA (Figure 4) (Park et al., 2013) and in β-oxidation it was produced from enoyl-CoA through (S)-3HA-CoA. However, it was concluded that the 3HB monomer was produced from acetyl-CoA and 3HA (3HV, when fatty acid was propionic acid) monomer was produced from other CoA sources such as 3HV from 3-ketovaleryl-CoA. The combination of 3HB and 3HV monomers produced a copolymer of poly(3HB-co-3HA) when fatty acids were used (Figures 4 and 5). As an example, efficient poly(3HB-co-3HV) accumulation from waste frying oil was produced by *Halomonas hydrothermalis* (Pernicova et al., 2019).

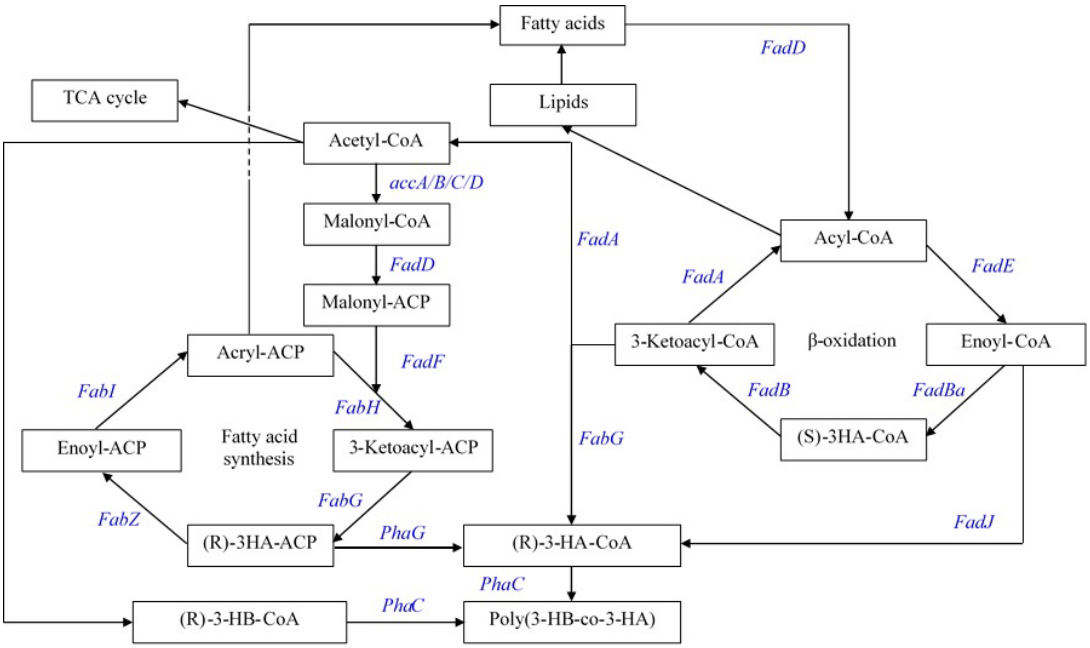

Figure 5. Metabolic network of pure culture from fatty acid for the production of poly(3HB) copolymer (Shimizu et al., 2013; Chen & Jiang, 2017; Mohapatra et al., 2017)

In PHA producing bacteria (mainly *C. necator*); the *accA1*, *accA2*, *accB*, *accC1*, *accC2*, *accC3*, and *accD* genes were the genes recognized to encoded for the subunits known as ACC subunits that formed carboxylase acetyl-CoA. The major ACC genes *accA1* and *accD* were encoded for the transferase carboxyl subunit α and carboxyl subunit β, respectively. The *accB* gene was encoded for the biotin carboxyl carrier protein and *accC2* for biotin carboxylase. The activities of these genes were very high in the condition of cells growth and low in the PHA production stage. The genes *fabZ*, *fabII*, which were involved *in situ* fatty acid production, were highly active in both cell growth and PHA production stages when fatty acids were used as the carbon source but showed low activities when carbohydrates were used (Shimizu et al., 2013). Simple fatty acids followed the described metabolism but in case of isocaproic acid the metabolic pathway was still unknown. Tanadchangsaeng et al., (2009) & Lau et al., (2010) proposed leucine pathway for isocaproic acid metabolism while the metabolism pathway of other branched chain fatty acids followed β-oxidation pathway (Massey et al., 1976).

The metabolic pathway of branched chain fatty acids was predicted to involve 3-keto-4-methylvaleryl-CoA production from 4-methylvaleryl-CoA and subsequently formation of R-3-hydroxy-4-methylvalery (3H4MV)-CoA. R-3H4MV-CoA was then polymerized to produce a copolymer with 3-hydroxy-4-methylvalery (3H4MV) monomer by the *phaC* enzyme. Furthermore, 3-keto-4-methylvaleryl-CoA could be divided into two CoAs; acetyl-CoA and isobutyryl-CoA. Propionyl-CoA was produced from isobutyryl-CoA following the isoleucine metabolic pathway and then 3-ketovaleryl-CoA produced from acetyl-CoA and propionyl-CoA. Generally, the 3HV monomer was produced from 3-ketovaleryl-CoA. The metabolic pathway for the production of the 3HV monomer was longer than that of the 3H4MV monomer and hence Chia et al. (2010) found relatively lower composition of the 3HV monomer (1%) compared to the 3H4MV monomer (3%) in PHA copolymers. It was shown that mcl-HA-CoAs was possible to be produced through the

metabolism of fatty acids via β-oxidation and *in situ* fatty acid production (de Smet et al., 1983; Andin et al., 2017). When fatty acids entered the β-oxidation pathway, R-3HA-CoAs with different carbon lengths were generated and subsequently polymerized into mcl-PHAs by *phaC*. As an alternative pathway, acetyl-CoA was converted into R-3HA-CoAs via the *de novo* fatty acid biosynthesis pathway, and thus mcl-PHAs could be produced. Extensive examination of different strains with genetic modification for the biosynthesis of mcl-PHA were conducted (de Smet et al., 1983; Rehm et al., 1998; Matsumoto et al., 2002; Ren et al., 2009; Galán et al., 2011). Molecular characterization of *Aeromonas caviae* identified that the key enzyme responsible for PHA production was *phaC*, which had a high carbon source specificity for 3HB-CoA, 3HHx-CoA, and R-specific-enoyl-CoA hydratase (*phaJ*), which was responsible for producing 3HB and 3HHx monomer units from their corresponding enoyl-CoA (Fukui & Doi, 1997; Fukui & Doi, 1998; Fukui et al., 2001). A number of enoyl-CoA hydratase genes were identified in *P. aeruginosa*, *P. putida* and strains of *Escherichia* and all of them were involved in mcl-PHA biosynthesis from fatty acids through β-oxidation pathway (Park & Lee, 2003). However, in a recent study of the production of PHA copolymers from levulinic acid using *P. putida* EM42 for an engineered metabolic pathway, scl-PHA comprising different monomers such as 3HB, 3HV and 4HV were produced. The genes involved in this metabolic process for the production of copolymer were heterologous PHA synthase (*phaEC*), acetyl-CoA acetyltransferase (*phaA*) and acetyl-CoA reductase (*phaB*) (Cha et al., 2020).

3.3 Mixed carbon sources

A number of studies were conducted for the production of PHA copolymers using mixed organic materials. In poly(3HB-co-3HV) copolymer, the 3HV fraction was controlled by controlling the feeding of propionic acid within a mixed carbon source (Byrom, 1990). Although some microorganisms, such as *Burkholderia* sp., were not grow on propionic acid, but were able to produce poly(3HB-*co*-3HV) from a mixture of carbohydrates and propionic acid (Silva et al., 2000). *Halomonas bluephagenesis*, a recombinant strain, demonstrated the ability to produce poly(3HB-*co*-3HV) copolymer from a mixture of glucose and gluconate (Chen et al., 2019b). To produce the 3HV monomer, the fatty acid predominantly followed α/β-oxidation pathway (Figures 4 and 5). The enzymes that were potentially functioning in fatty acid oxidation were also responsible for the degradation of hydrophobic compounds, and the activities of some of them increased during PHA production phase compared to the growth phase. Similar activities were reported when trioleate was used as carbon source (Brigham et al., 2010) but were moderately expressed when fructose was used. These results revealed that in the presence of fructose, fatty acid oxidation was conducted and produced copolymer even from a mixture of fatty acids or other hydrophobic carbon sources. The well-known PHA producing microorganisms such as *C. necator* showed relatively high activities towards 3HB-CoA, compared to other PHA monomer-CoAs such as 3HP-CoA, 4HB-CoA, and 2HB-CoA (Yuan et al. 2001; Zhang et al., 2001). As described in Figure 6, PHA copolymers were produced from various mixtures of carbohydrates that produce corresponding hydroxycarboxylic acids as monomer component of PHAs. By combining all the metabolic processes outlined in Figure 6 in a single organism, the copolymer poly(3HB-co-3HV-coLA-co-3HHX) should be the result. The monomer composition of a copolymer depends on the presence of enzymes and metabolic processes conducted in the organism to assimilate the available carbon sources. Different organisms had different levels of gene activities towards different carbon sources but could produce copolymers from mixed substrates (Ghysels et al., 2018). Poly(3HB-co-3HV) was produced either from a mixture of glucose and propionic acid or a mixture of

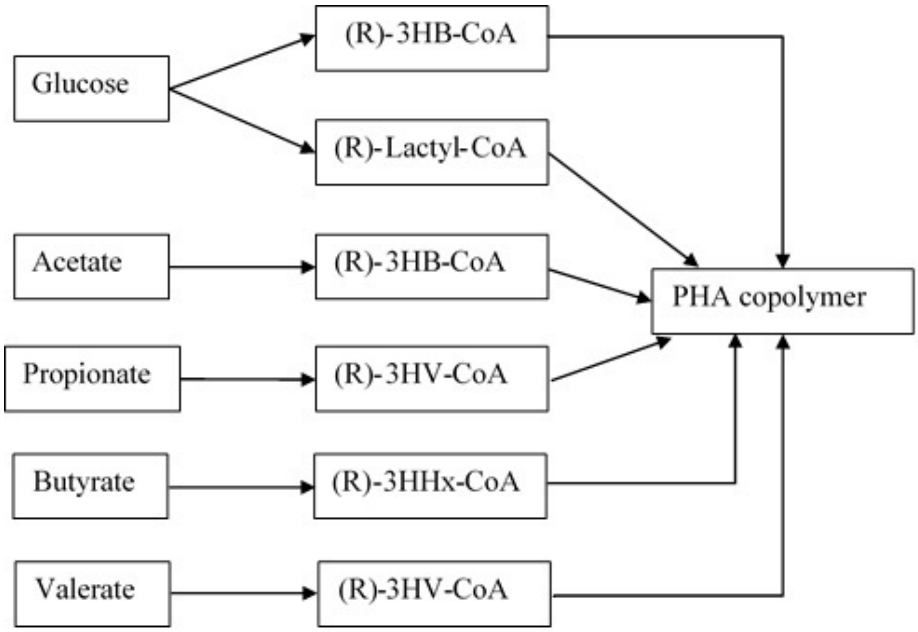

Figure 6. Synthesis of PHAs copolymer produced from mixed substrate

glucose and valeric acid (Policastro et al., 2021). The activity analysis of PHA producing organisms and their genes/enzymes showed that most of the organisms were not able to produce polylactic acid (PLA) or its copolymer; poly(3HB-co-LA). Making a mixed culture of recombinant *E. coli* with other microorganisms that converted glucose to lactate produced a copolymer with lactate; poly(3HB-*co*-LA). The recombinant *E. coli* followed an engineered metabolic pathway in which the natural pathway pyruvate to acetyl-CoA was deleted and new pathway was established and amplified for the conversion of lactyl-CoA to lactate monomer (Yang et al., 2018). In this engineered metabolic process, the genes *ackA* and *adhE* were removed and the *acs* gene promoter was replaced by the *trc* promoter that enhanced the acetyl-CoA concentration with excess acetyl-CoA being donated to CoA to produce D-lactyl-CoA. Removal of *ldhA* gene promoter and *ppc* gene and introducing *trc* gene also enhanced the lactate production from pyruvate. Recombinant *H. bluephagenesis* produced PHBHHx copolymer from glucose and sodium hexanoate (Wang et al., 2023a). In another way, it was also possible to produce homopolymers from mixed substrates. For example, polyhydroxybutyrate was produced from a mixture of glucose, xylose, and lignin-derived aromatics using engineered *C. necator* (Weng et al., 2023) due to the presence of specific enzymes in this engineered strain. One of the strategies to produce a new copolymer is to produce an engineered strain with the desired genes. A broad range of carbon sources were used to produce different CoA derivatives through different CoA-transferases that had been engineered with the desired genes. The CoA derivatives were converted to the corresponding monomers and then into PHA copolymers of the desired composition of monomers by capable PHA synthase. This system also had the potential to produce more diverse copolymers with desired monomer compositions. Engineered strains were shown to be able to control the metabolism specifically towards PHA synthesis by suppressing other metabolic branches that enhanced PHA production (Wang et al., 2023b).

3.4 Inorganic carbon sources

Hydrogen oxidizing bacteria were able to produce energy for self-maintenance from CO_2 when H_2 was used as electron donors. This reduction process of CO_2 followed the acetyl-CoA pathway. These organisms reduced CO_2 to PHAs via the Wood-Ljungdahl pathway, which differentiated them from other PHA producing organisms. The intracellular concentration of phosphoenolpyruvate became low under autotrophic conditions (Shimizu et al., 2013); therefore, under autotrophic (using CO_2 as carbon source) conditions, different pathway compared to heterotrophic (using organic carbon source) condition were followed. The designed metabolic pathway of autotrophic PHAs production, summarized from the proposed metabolic pathway by Hu et al. (1984); Martin & Russell (2006); Ragsdale (2008) and Kung et al. (2012), is shown in Figure 7. In this metabolic pathway, CO_2 was reduced through two different active sites. At one active site, three hydrides (H) was transferred to reduce CO_2 and produced cofactor-bound methyl (N5, N10-methyl-FH4) complex with the support of a molybdenum cofactor and the formate dehydrogenase enzyme. A number of steps were involved in this CO_2 reduction process. At first CO_2 was converted to formate with the consumption of a hydride (H) and then to formyltetrahydrofolate (formyl-FH4) through consumption of ATP. Formyl-FH4 was then converted to methenyl-FH4 and subsequently to methylene-FH4 and methyl-FH4 complex using two hydrides (H) in last two steps. At the end of the circle, a methyl group was transferred by methyl-transferase enzyme to the corrinoid iron-sulfur protein (CoFeSP) to produce methyl-CoFeSP (Müller, 2003), liberating tetrahydrofolate (FH4) that was then recycled to take part in formyl-FH4 formation.

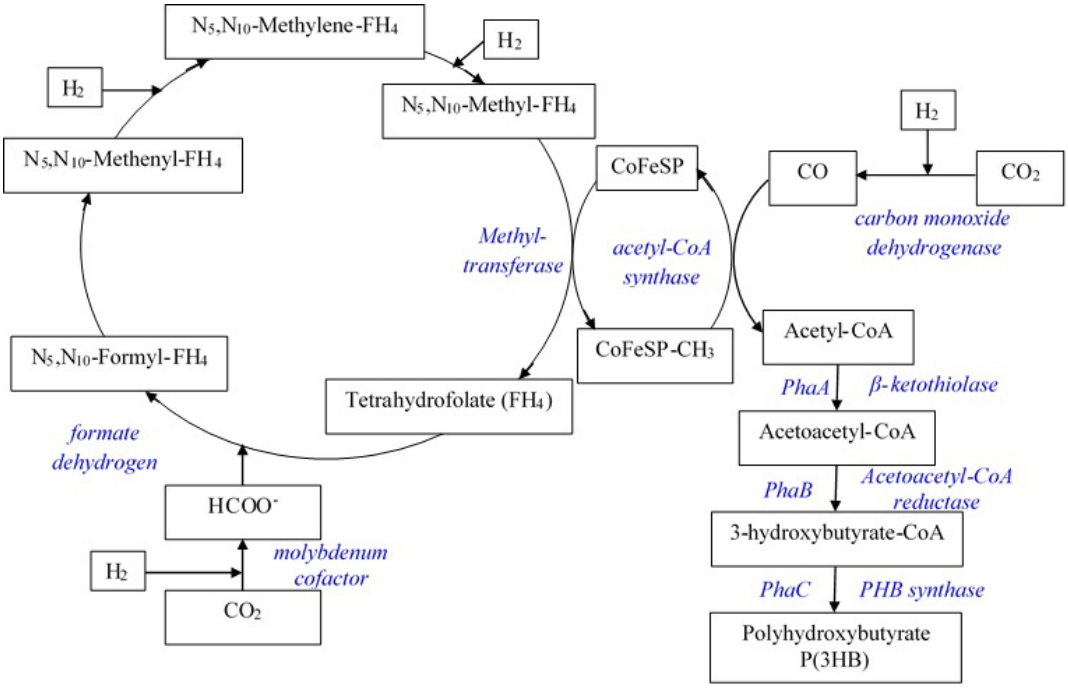

Figure 7. Metabolic network for the production of poly(3HB) over CO_2 (Hu et al., 1984; Martin & Russell, 2006; Ragsdale, 2008; Kung et al., 2012)

At another active site, CO_2 was reduced to CO through two electron transfer from H2 (equation 1). Under autotrophic conditions, the enzyme CO dehydrogenase (CODH) was more active and the cluster of CODH/acetyl-CoA [Ni4Fe5S] acted as an enzyme in biosynthesis of acetyl-CoA from CO. [Ni4Fe5S] bought CO and made a bond with the Ni-Ni-[4Fe4S] cluster enzyme (Volbeda & Fontecilla-Camps, 2006). A methyl group from methyl-CoFeSP was transferred to Ni–Ni–[4Fe4S] enzyme cluster and a bond was made with CO bearing Ni (Doukov et al., 2002; Lindahl, 2002; Darnault et al., 2003; Svetlitchnyi et al., 2004), producing metal-bound acetyl and subsequently acetyl-CoA. In the acetyl to acetyl-CoA production step, the acetyl was separated from the metal bond by reaction with free thiol CoA (CoASH) (Lindahl, 2002; Svetlitchnyi et al., 2004; Volbeda & Fontecilla-Camps, 2006). Overall, Ni4Fe5S catalyzed the reaction of equation 1 and Ni–Ni–[4Fe4S] coupled the reaction of equation 1 and equation 2 to release acetyl-CoA.

$$CO_2 + H_2 \rightarrow CO + H_2O \quad (1)$$

$$CO + CH_3\text{-CoFeSP} + CoA \rightarrow \text{acetyl-CoA} + CoFeSP \quad (2)$$

The production of PHAs from acetyl-CoA under autotrophic process was mainly in three steps that were conducted with help of three different enzymes and their encoded genes. In first step, acetoacetyl-CoA was produced from two acetyl-CoA; the enzyme 3-ketothiolase and corresponding gene *phaA* were responsible for this bioconversion. Depending on NADPH and the *phaB* gene, acetoacetyl-CoA reductase biosynthesized 3-hydroxybutyryl-CoA from acetoacetyl-CoA (Steinbüchel & Schlegel, 1991). In the last step, PHA synthase by *phaC* gene polymerized 3-hydroxybutyryl-CoA producing poly(3HB) (Stubbe & Tian, 2003; Garcia-Gonzalez et al., 2015).

4. How are the Properties of PHA Regulated by Carbon Source?

The application of PHAs was mainly governed by the properties of the produced PHAs which were determined by the process conditions as well as carbon sources and organisms. Pure poly(3HB) is brittle, but its homo or copolymers have shown to possess a wide range of properties that attract a lot of industrial attention. It was possible to produce PHA homo or copolymers through regulating carbon sources or their feeding. Carbon sources and organisms determined the rate of polymerization and molecular weight distribution that influenced the chemical, physical and thermal properties of the PHA homo and copolymers (Donaruma, 1991), which are summarized in Table 1. The copolymers of the PHAs had different monomer composition and side chain(s) that influenced the chemical, physical, thermal and mechanical properties of the PHAs.

4.1 Chemical properties

The production of PHA homo or copolymers was mainly governed by available carbon sources. As an example, homopolymer poly(3HB) was produced from pure oleic acid (OA) while a mixture of hexanediol and butanediol with oleic acid led to the production of the copolymer poly(3HB-co-4HB) (Table 1) (Iqbal & Amirul, 2014). Volatile fatty acids including acetate, propionate and butyrate as carbon sources produced homopolymer poly(3HB); however, with this mixture the organism preferentially assimilated butyrate first (Pu et al., 2020). A mixture of acetic acid and valeric acid produced the copolymer poly(3HB-co-3 HV) and the organism *Bacillus* sp. CYR1 utilized a higher portion of acetic acid compared to valeric acid. This organism was unable to utilize higher chain fatty acids (Reddy et al., 2020). Copolymers of novel composition, poly(3HB-co-3HV-co-3H4MV-co-3HHx) and

poly(3HB-co-3H4MV-co-3HHx), were produced using a mixture of crude palm kernel oil and isocaproic acid. The fraction of 3H4MV and 3HV depended on the isocaproic acid concentration. However, increasing the concentration of isocaproic acid decreased biomass concentration as well as PHA content (Chia et al., 2010). Poly(GL-co-3HB) was produced through simultaneous feeding of glycolate (GL) and xylose as carbon source and *E. coli* as organism. The molar ratio of GL in the copolymer was controlled by glycolate concentration in the medium (Matsumoto et al., 2017). Matsumoto et al. (2018) produced block copolymers of poly(2HB-co-3HB) from glucose and sodium (R,S)-3HB and sodium (R,S)-2HB using engineered *E. coli*. The block copolymer produced from two different carbon sources indicated the organism's ability to swiftly switch between the two carbon sources during polymerization process.

4.2 Molecular weight and polydispersity index

The molecular weight of produced PHAs were strongly influenced by the microorganism (Donaruma, 1991; Hsieh et al., 2009; Chia et al., 2010), culture conditions (Taidi et al., 1994; Cavalheiro et al., 2012) and carbon sources (Donaruma, 1991; Hsieh et al., 2009; Chia et al., 2010). Besides fermentation conditions, culture time, pH, and aeration had great influence on molecular weight of PHAs (Sudesh et al., 2000; Lee et al., 2004). Generally, PHA homopolymers were produced from single carbon sources and natural organisms. The average molecular weight (Mw) of the PHAs varied from 1457 to 505 kDa (Table 1) and this variation was mainly due to the choice of carbon source. Kucera et al. (2018) studied the effects of the presence of NaCl in the fermentation medium on the Mw and polydispersity index (PDI). Both increased with increasing NaCl concentration. Copolymers were produced from mixed carbon sources and PHA contents as well as the production capacity of the cells did not affect the molecular weight of copolymers. The molecular weight depended on the monomer composition of the copolymer, which was controlled by the carbon source and feeding behavior. For example, molecular weight decreased with increasing 4HB fraction in copolymer poly(3HB-co-4HB) (Huong et al., 2014). The activity rate of PHA synthesis had a great influence on copolymer molecular weight. High PHA synthesis activities increased the number of chain ignition events, resulting in shorter polymer chains as well as low molecular weight PHAs (Huisman et al., 1992; Sim et al., 1997; Nomura & Taguchi, 2007; Tsuge et al., 2007). Higher carbon source specificity and activity also increased the initiation of monomers that decreased the molecular weight. Iqbal & Amirul (2014) found a decreasing trend of molecular weight of copolymer poly(3HB-co-4HB) with increasing 1,6-hexanediol in the fermentation medium. When using fatty acid as carbon sources, the Mw of PHAs depended on the number of double bonds in the fatty acid molecules and their chain lengths. PHA synthesis activity decreased with decreasing double bond in the carbon source and decreased with increasing chain length. This indicated that the type and concentration level of carbon sources played an important role in the determination of molecular weight. However, no significant difference in molecular weight was observed by Al-Kaddo et al. (2020) with the changing 4HB fraction in poly(3HBco-4HB) copolymer that had been produced by Cn-Kad1 from sodium-4HB. However, poly(3HBco-4HB) from two different carbon sources showed two different Mw characteristics.

The polydispersity index is an important characteristic of polymers that expresses the uniformity of polymer molecules in the polymer matrix. Iqbal & Amirul (2014) showed that the polydispersity index (PDI) was increased from 1.87 to 5.19 by increasing the 4HB fraction from 0 to 39% in the copolymer of poly(3HB-*co*-4HB); meaning number average molecular weight decreased at a high rate compare to weight average molecular weight. Further increasing the 4HB fraction caused a sharp decrease in the PDI, and for 47% 4HB content, it was 1.74. The random distribution of 3HB and 4HB monomers in poly(3HB-co-

4HB) copolymers were the main reason for changing PDI with monomer composition. But Al-Kaddo et al. (2020) found no significant difference in the polydispersity (1.6 to 1.9) of poly(3HB-co-4HB) copolymer with variation of monomers composition. Copolymer produced from a single carbon source had more homogenous polymer chains and relatively low polydispersity index (Table 1).

4.3 Crystallinity

Pure poly(3HB) is highly crystalline, stiff and brittle. The crystallinity of poly(3HB) was decreased by introducing other monomers such as 3HV and 3HHx (Volova et al., 2013) (Table 1). The copolymer poly(3HB-co-3HV) showed lower crystallinity as well as lower melting point compared to pure poly(3HB) (Donaruma, 1991; Holmes, 1985, 1988). Using only oleic acid produced pure poly(3HB) of 47.3% crystallinity. Mixing oleic acid, hexanediol and butanediol increased the 4HB monomer from 0 to 65% in poly(3HB-co-4HB) copolymer and decreased the crystallinity from 47.3% to 4.1% (Iqbal & Amirul, 2014). This was due to the longer chain of the 4HB monomer compared to the 3HB monomer, which reduced the co-crystallization behavior of 4HB in the poly(3HB) lattice. The 4HB monomer fraction in the homopolymer of poly(3HB) converted the crystalline polymer to a strong elastomer (Al-Kaddo et al., 2020). Iqbal & Amirul (2014) did not find any crystallinity for 39% and 47% 4HB content in the poly(3HB-co-4HB) copolymer, indicating that these copolymers were in an amorphous state. The introduction of 3H4MV and 3HHx monomers in the production of the copolymer poly(3HB-co-3HV-co-3H4MV-co-3HHx) produced lower crystallinity although the backbone of the polymer was made of 3HB monomers (Chia et al., 2010). Increasing glycolate concentration with xylose increased the GL monomer fraction in copolymer of poly(3HB-co-GL), significantly reducing crystallinity. Matsumoto et al. (2017) found 54% crystallinity for pure poly(3HB) when it was produced from only xylose. However, the crystallinity decreased to 4% when xylose was mixed with 10 g/L glycolate which introduced 15% GL monomer into the copolymer (Table 1). Interestingly, process conditions had a significant effect on the determination of crystallinity of poly(3HB). The presence of NaCl in the fermentation medium had a substantial effect of the crystallinity of poly(3HB). Besides the composition and monomer fractions in PHAs, the purity of the product and process conditions were influential factors for the determination of molecular weight as well as crystallinity of the produced PHAs. Low molecular weight and low polydispersity index were also indications of low crystallinity (Kucera et al., 2018). However, when comparing different copolymers, Kiselev et al. (2022) found lower crystallinity with the incorporation of 4HB monomer into the polymer compared to those seen when 3HV and 3HHx monomers were incorporated.

4.4 Mechanical properties

Poly(3HB) homopolymers demonstrated high tensile strength and Young's modulus but lower elongation-to-break compared to their copolymers. On the other hand, poly(4HB) homopolymer was very strong; its tensile strength was similar to polyethylene. It had high elastic properties that were confirmed by 100% elongation-to-break tests (Martin & Williams, 2003).

The molecular weight distribution has a great effect on mechanical properties; high molecular weight PHAs were elastomers while those of low molecular weight were soft and sticky. Moreover, the elongation-to-break decreased if one of the monomer fractions became higher in the copolymer. Monomer fraction was controlled by substrate. Oleic acid produced pure poly(3HB) and mixing oleic acid with hexanediol and butanediol increased the 4HB monomer from 0 to 65%, which had a significant effect on elongation-to-break. For instance, elongation-to-break increased with increasing 4HB fraction of upto 30% in

poly(3HB-co-4HB) and then decreased (Iqbal & Amirul, 2014). Increase of the monomer composition of 3H4MV and 3HHx in poly(3HB-co-3HV-co-3H4MV-co-3HHx) by using crude palm kernel oil with isocaproic acid decreased tensile strength and Young's modulus but increased elongation-to-break (Table 1). Incorporation of the 3H4MV and 3HHx monomers into poly(3HB) reduced its rigidness but increased its elongation-to-break and ductility (Chia et al., 2010). The elasticity and the flexibility of poly(3HB-co-3HV-co-3H4MV-co-3HHx) were due to the combined effect of both the 3H4MV and 3HHx monomers. Increasing glycolate concentration with xylose increased the GL monomer in poly(GL-co-3HB), reducing Young's modulus and increasing elongation-to-break. The GL monomers were also responsible for an increase in the stretchiness of the homopolymers of poly(3HB) (Matsumoto et al., 2017). Overall, copolymer exhibited high flexibility as well as low crystallinity. Based on commercial interests, it is also possible to better target the properties and biocompatibility of PHAs by blending them with other polymers, mixing with other inorganic materials and by modifying their surfaces.

4.5. Thermal properties

The thermal characterizations of each polymer and copolymer of PHAs are described in Table 1. Poly(3HB) homopolymer showed the maximum melting points (T_m) and glass transition temperature (T_g). Reduced thermal properties were observed with incorporation of other monomers into poly(3HB). The 3HB monomers in the backbone of copolymer were deformed by the stearic interference of the carbonyl oxygen of co-monomers on the methyl side chains of poly(3HB) and responsible for lowering melting and glass transition temperature (Madden et al., 2000). The GL monomer produced from glycolate in poly(GL-co-3HB) copolymer showed low melting point but there was no straight forward relation between the type of polymer or copolymer and the decomposition temperature (T_d) (Matsumoto et al., 2017). Table 1 revealed a wide variety of T_d for their homo or copolymers that were in the range of 224 to 310°C. However, Volova et al. (2013) concluded that a straight forward relationship; the melting point and thermal degradation temperature decreased with decreasing molecular weight if the monomer composition was the same. Poly(3HB-co-4HB) copolymer produced from the mixture of oleic acid, hexanediol and butanediol showed lower melting and glass transition temperature compared to pure poly(3HB) from oleic acid. Increasing 4HB monomer in poly(3HB-co-4HB) copolymer created interference in the poly(3HB) crystal, reducing the melting and glass transition temperature as well as crystallinity (Iqbal & Amirul, 2014) (Table 1). Increasing branching or sidechains in PHA polymers lowered Tm but polar sidechains or sidechain with steric forces increased the Tm. The presence of NaCl in fermentation medium had a significant effect on the thermal properties of PHAs (Kucera et al., 2018). High NaCl concentration reduced the thermal stability of poly(3HB), and Kucera et al. (2018) determined its melting point to be about 180°C, which was almost the same as the melting point of poly(3HB) of high molecular weight (Koller et al., 2017). Copolymers with monomers containing higher levels of sidechains had improved mechanical properties such as lowering brittleness, reducing crystallinity, decreasing glass transition temperature, and increasing melting temperatures. Higher levels of side chains also reduced the tensile strength of the polymer. The difference between melting and degradation temperature of greater than 100°C indicated the thermal stability of PHAs. Thermal stability increased with increasing 4HB monomer in poly(3BH) (Table 1) (Hu et al., 1984). The differences in thermal stability of homo or copolymers were also related to the degree of crystallinity.

Table 1. Typical properties of homo and copolymer of PHAs and their source organisms and carbon sources

Carbon source	Organism	Type of PHA	M_w (kDa)	PDI	Tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)	T_g (°C)	T_m (°C)	T_d (°C)	Crystallinity (%)	Reference
Sodium-4-HB (0.1-0.4 M)	*Burkholderia contaminans* Kad1 *phaC* gene into *C. necator*	Poly(3HB-co-28%4HB)	800±10	1.6				-30	162		3.9	(Al-Kaddo et al., 2020)
		Poly(3HB-co-30%4HB)	910±20	1.8				-32	160		1.9	
		Poly(3HB-co-34%4HB)	1010± 10	1.7				-34	159		1.3	
		Poly(3HB-co-54%4HB)	880±30	1.9				-35	155		0.27	
Xylose (20 g/L) + Glycolate (0-10 g/L)	*Escherichia coli*	Poly(3HB)	400	3.6	29 ± 2	1620 ±97	15±1	1.1	173		54.3	(Matsumoto et al., 2017)
		Poly(5%GL-co-95%3HB)	330	4.3	25 ± 3	806 ± 90	9 ± 4	3.0	154		33	
		Poly(9%GL-co-91%3HB)	290	4.2	17 ± 1	400 ± 21	19 ± 4	3.9	149		20.3	
		Poly(11%GL-co-89%3HB)	110	2.5	11 ± 2	132 ± 29	37 ± 7	2.7	142		7.7	
		Poly(15%GL-co-85%3HB)	150	4.0	7 ± 1	54 ± 9	99 ± 7	4.1	137		3.6	
Glucose	*Cupriavidus necator* DSM545	PHB	1457	1.18				-6.6	142	270	52	(Garcia-Gonzalez et al., 2015)
Waste glycerol		PHB	992	1.59				-	168	250	55	
CO_2		PHB	625	2.03				-	-	-		
CO_2		PHB	1222	1.64				-1.2	159	255	58	
CO_2		PHB	749	1.36				-4.5	141	280	62	
Oleic acid (OA)	*Cupriavidus* sp. USMAA2-4	Poly(3HB)	505±13	1.87	24±2	192±11	4±1	2.5	170	290	47.3	(Iqbal & Amirul, 2014)
60% OA + 40%1,4-Bol		Poly(3HB-co-10%4HB)	342±16	2.63	15±1	66±3	60±4	-12	147	295	31.6	
34% OA + 49%1,6-Hol + 17%1,4-Bol		Poly(3HB-co-21%4HB)	375±5	3.1	8±1	53±8	379±21	-16	132	290	18.2	
24% OA + 63%1,6-Hol + 13%1,4-Bol		Poly(3HB-co-30%4HB)	376±10	3.55	5	43±4	368±14	-17	121	300	12.9	
5% OA + 73%1,6-Hol + 21%1,4-Bol		Poly(3HB-co-39%4HB)	330±14	5.19	2	24±2	156±3	-23	-	300	-	
86%1,6-Hol + 14%1,4-Bol		Poly(3HB-co-47%4HB)	51±7	1.74	3	20±2	27±3	-30	-	310	-	
99%1,6-Hol + 01%1,4-Bol		Poly(3HB-co-65%4HB)	47±3	1.82	2	13±1	14±2	-31	68	300	4.1	

Table 1. Typical properties of homo and copolymer of PHAs and their source organisms and carbon sources (continued)

Carbon source	Organism	Type of PHA	M_w (kDa)	PDI	Tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)	T_g (°C)	T_m (°C)	T_d (°C)	Crystallinity (%)	Reference
Glucose	*Pseudomonas aeruginosa*	Poly(3HV-co-5HDE)	6	1.06				34.3± 1.2	139± 2.3	225± 4	32.2±2.3	(Phukon et al., 2013)
CO_2	*Cupriavidus eutrophus* B-10646	Poly(3HB-co-0.9%3HV)	922	2.51					179	294.8	76	(Volova et al., 2013)
CO_2 + Butyrolactone		Poly(3HB-co-37%4HB-co-0.3%3HV-co-0.3%3HHx)	537	3.44					170.5	287	36	
CO_2 + Valerate		Poly(3HB-co-65%3HV)	1111	3.49					153	249	53	
CO_2 + Hexanoate		Poly(3HB-co-1%3HV-co-15%3HHx)	924	4.1					174.8	283.8	60	
		Poly(3HB)			40	3.5	5	4	180			(Akaraonye et al., 2010)
		Poly(4HB)			104	149	1000	-48	53			
		Poly(3HB-co-20%HV)			20	1.2	50	-1	145			
		Poly(3HB-co-16%4HB)			26	-	444	-7	150			
		Poly(3HB-co-10%HHx)			21	-	400	-1	127			
		Poly(3HB-co-6%3HD)			17	-	680	-8	130			
Crude Palm Kernel Oil + isocaproic acid (0-1 g/L)	*Cupriavidus necator*	Poly(3HB-co-5%HHx)	635	3.6	36±3	1179±221	12±2	-0.6	129	285		(Chia et al., 2010)
		Poly(3HB-co-1%3H4MV-co-10%HHx)	1058	2.5	27±4	623±223	84±12	1.4	118	270		
		Poly(3HB-co-2%3H4MV-co-12%HHx)	771	2.2	24±1	499±42	367±196	-0.2	117	265		
		Poly(3HB-co-1%3HV-co-3%3H4MV-co-18%HHx)	701	2.4	15±1	84±14	478±57	-1.9	113	290		

Table 1. Typical properties of homo and copolymer of PHAs and their source organism and carbon source (continued).

Carbon source	Organism	Type of PHA	M_w (kDa)	PDI	Tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)	T_g (°C)	T_m (°C)	T_d (°C)	Crystallinity (%)	Reference
Glucose	*Bacillus megaterium* IOU303A	Poly(3HB-co-2.5%3HV)			42	3.3	42	8.5	178.1		48.4	(Reddy et al., 2009)
Glycerol		Poly(3HB-co-5%3HV)			40	3.5	5	8.8	175.2		50	
Acetate		Poly(3HB)			33	3.1	19	8.2	174.3		44.1	
Lauric acid + 1,4-Bol (0-25 g/L)	*Aeromonas hydrophila* 4AK4	Poly(3HB)	610	1.75	18.5	1510	4.45	5.4	165	235		(Xie & Chen, 2008)
		Poly(3HB-co-7%4HB)	680	1.62	11.5	480	48.2	-6.69	114	243		
		Poly(3HB-co-12%3HHx)	440	2.03	4.5	135	108	-1.75	94	239		
		Poly(3HB-co-4.3%4HB-co-22%3HHx)	666	1.5	0.62	3.8	504	-9.34	-	253		
		Poly(3HB-co-8.5%4HB-co-17.6%3HHx)	699	1.41	0.21	2.49	110	-9.2	-	249		
Dodecanoic acid + propionic acid (0-30 g/L)	*Aeromonas hydrophila* 4AK4	Poly(3HB)	613	1.75	18.5	1510	4.45	-1.18	162	226.6		(Zhao & Chen, 2007)
		Poly(3HB-co-5%3HV)	-	-	2.4	62.65	123	-1.73	156	223.8		
		Poly(3HB-co-12%3HHx)	440	2.03	4.5	135	108	-1.75	97	239.4		
		Poly(3HB-co-1.2%3HV-co-15.1%3HHx)	528	1.81				-1.79	104	248.5		
		Poly(3HB-co-5.4%3HV-co-9.9%3HHx)	373	2.17				-2.59	129	273.1		

GL= glycolate, OA= oleic acid, Hol= hexanediol, Bol= butanediol, PDI=polydispersity index, HDE=hydroxydecenoare, HB=hydroxybutyrate, HV=hydroxyvalerate, HHx=hydroxyhexanoate, 3H4MV=3-hydroxy-4-methylvalerate, Mw = average molecular weight

4.6. Biodegradability

One of the most extensive properties of PHAs are biocompatibility and biodegradability. A number of organisms available in nature can degrade PHA homo or copolymers either under aerobic or anaerobic conditions. In biodegradation process, PHAs were first depolymerized and hydrolyzed to produce oligomers and then to monomers through the action of specific enzymes; at the end, carbon dioxide and water were produced (Jendrossek & Handrick, 2002; Lim et al., 2005; Sridewi et al., 2006). The biodegradability of PHAs has been correlated with their physical and chemical properties. Or example, biodegradability decreased with increasing melting temperature. The chemical composition of PHAs was also a determining factor of their biodegradability; presence of 4HB monomer increased the degradation rate compared to poly(3HB) and poly(3HB-co-3HV) and 4HB monomer in copolymer was increased in a mixed substrate, e.g. oleic acid mixed with hexanediol and butanediol (Iqbal & Amirul, 2014), and lauric acid with butanediol (Xie & Chen, 2008). Volova et al. (2003) proved that poly(3HB) and poly(3HB-co-3HV) did not show any toxicity to any living organisms. Saito & Doi (1994) also found that poly(3HB) did not cause any inflammation in living organisms. PHAs are degradable in nature although it varied based on microbial population in soil, temperature, pH, and soil moisture (Fernandes et al., 2020). The biodegradation of PHAs was also affected by the functional groups present in the polymer, molecular hydrophilicity-hydrophobicity balance, the orientation of monomers and morphological properties. High order structures displayed high crystallinity and had low biodegradability (Nishida & Tokiwa, 1993). Higher crystallinity led to less amorphous regions that organisms could attack, reducing the degradability (Abe & Doi, 1999). The hydrolytic degradation of poly(GL-co-3HB) copolymer increased with the existence of GL monomers that were present with increasing glycolate concentration as the co-substrate (Matsumoto et al., 2017) and hence increased the biodegradability of poly(GL-co-3HB). The biodegradation rates of poly(3HB-co-3HV) and poly(3HB-co-3HHx) increased under marine conditions and this was related to the crystallinity of the polymer (Volant et al., 2022).

5. Conclusions and Future Perspectives

The metabolic reactions within the selected organisms that depended on the carbon sources and enzymes present in the organisms determined the homo/copolymer of the PHAs. Different carbon sources followed different metabolic pathways within the same organisms and thus produced various PHA copolymers. The type of carbon source played a major role in determination of the PHA homo/copolymers. Pure hydrocarbons and natural organisms produced poly(3HB) homopolymers, but mixed carbohydrates produced copolymers. Fatty acids produced either homopolymers or copolymers depending on the nature of the fatty acid. The composition of the monomers as well as the properties of PHAs were possible to be controlled through control of the carbon sources. It was possible to produce new diverse and desired copolymers with targeted properties by engineered strains with the desired genes. Homopolymer-based PHAs with high ordered structures showed high crystallinity and low amounts of amorphous regions that led to high tensile strengths and low biodegradability. The crystallinity of PHAs was reduced and biodegradability increased by introduction of glycolate (GL) or 4HB units in poly(3HB). Overall, PHAs with diverse physical properties increased application potentiality. However, lack of combination of desired monomeric compositions made their properties inappropriate for target applications. Alteration of substrates and enzyme modification are promising subjects for further research, the aim of which should be to reduce undesired properties as well as enhance desired properties. Enzymatic-catalyzed methods can be one of the alternatives, but further development is needed. In the future, it is expected that

further research involving alternative substrates and enzymes will lead to the PHA production of the desired properties and can be used in a range of applications.

6. Acknowledgments

The authors gratefully acknowledge the Bangladesh Bureau of Educational Information and Statistics (BANBEIS), Bangladesh for financial support to conduct this research (Project ID: SD20211542).

7. Conflicts of Interest

The authors declare no conflicts of interest.

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