The role of PHA transformation in obtaining distinct bioproducts: insights into biorefinery and medical applications

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Abstract- Since polyhydroxyalkanoates (PHAs) can be obtained by utilizing organic waste as a substrate, they form one of the most suitable classes of organic acids with many applications, ranging from medical supplies to energy and biorefinery products. In total, PHAs are found in at least in 150 different molecular forms, with the basic differences determined by the length of the carbon skeleton (4 to 14 C-bonds) and the radicals, which are formed by many organic groups. These characteristics make up their unique physicochemical properties, biodegradability and nontoxicity. Many new approaches are now being developed to obtain PHAs and modify these molecules into new green chemicals as well as renewable alternatives for petroleum and other non-sustainable compounds. This mini review gives insight into the genetic characteristics of PHA synthases, the most important enzyme group related to this compound. This review addresses chemical techniques to modify PHAs to obtain compounds with a high market value. Therefore, this work shows new applications and possibilities for PHA, such as drug-delivery systems, building block chemicals, new polymeric structures for tissue scaffolds and certain energetic compounds, such as n-butanol..

Index Terms- Polyhydroxyalkanoates, polyhydroxybutyrate, biorefinery, bioproducts.

I. INTRODUCTION

Polyhydroxyalkanoates (PHAs) are a class of organic compounds that occur naturally in bacteria, serving primarily as a carbon reserve in environmentally and metabolically unfavorable situations[1]. Usually, the PHA production process in the cells is attributed to factors such as an excess of carbon sources, the absence or depletion of nitrogen, phosphorus or other essential nutrients and even as a result of physiological stress[2, 3]. They are all biodegradable, nontoxic, water insoluble and are renewable[4], and at least 150 forms of monomeric units of these polyesters can be found [5]. Additionally, PHAs have unique physicochemical characteristics, such as a low glass transition temperature (Tg), high melting temperature (Tm) and tensile strength[6].

Currently, the elevated production and extraction costs and the use of chlorinated solvents are the major difficulties for using PHAs as a substitute for plastics. There are new alternatives for extraction that use nontoxic solvents, but they are expensive in most cases and difficult to employ at a large scale. Thus, the total amount of waste generated in this process of extraction could be a secondary problem, leading to cost increases for the final product[7-9].

Considering those situations (high costs to produce, extraction and substrate problems), many works are suggesting employing PHAs for other uses besides the production of petroleum-derived plastics, such as medical applications[10, 11], drug delivery and targeting[11], synthetic drugs[12] and even for obtaining new materials for industry[13].

This review provides a comprehensive vision of in vivo and in vitro transformations of PHAs into new bioproducts. There are a wide range of applications and forms of presentation for PHAs, including several economic aspects about the raw materials to obtain biopolymers.

reference of already accomplished work as a starting building block of its paper.

II. OBTAINING PHAS: FROM GENE MANIPULATION TO REACTOR DESIGN

The natural conversion of PHAs into bioproducts occurs in two types of processes, in vivo and in vitro. The in vivo approaches to convert PHAs into bioproducts involve several key points and strategies, depending on the focus of the PHA type. There are common techniques to manipulate PHA production when a specific type of HA is desired: strain selection, i.e., choosing a strain that produces several or only one HA type[14]; genetic transformation, i.e., inserting, modifying or blocking specific enzymes or metabolic pathways[15]; and feeding strategies, i.e., modifying the substrate or cultivation to increase the production[16, 17].

a. PHA SYNTHASES: INFLUENCE OVER PRODUCTION OF BIOPOLYMERS

According authors[6], PHA synthesis can involve at least 45 enzymes and 12 known routes. Doudoroff and Stanier[18] studying poly-3-hydroxybutyrate (PHB), one of the most common PHAs, found a metabolic route that uses acetyl-CoA and at least 3 enzymes: PhaA (β-ketoacyl-CoA thiolase), PhaB (acetoacetyl-CoA NADPH-dependent reductase or dehydrogenase) and PhaC (PHA synthase), as exemplified by Figure 01. Since acetyl-CoA, FADH and FADPH are the key resources in PHA formation, other metabolic routes that use those substrates will interfere with PHA biosynthesis[19, 20].

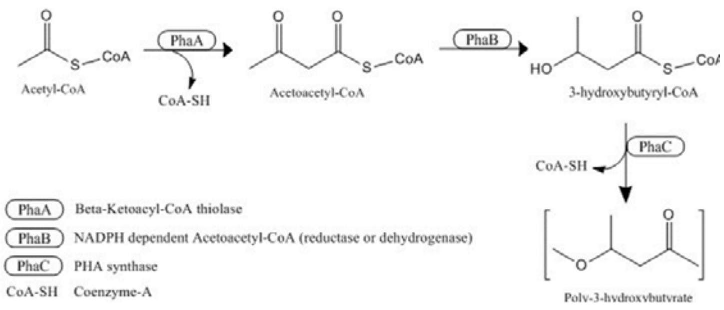

Figure 01. Overview of the most common route for PHB accumulation in bacteria, with the 3 main enzymes (PhaA, PhaB, PhaC) and the intermediary molecules generated. The process starts with the condensation of two molecules of acetyl-CoA into acetoacetyl-CoA by the action of PhaA. PhaB converts acetoacetyl-CoA, formed previously, into 3-hydroxybutyryl-CoA, which will be polymerized by PhaC by removing the coenzyme-A.

Each metabolic pathway has specific enzymes and activities with HA monomers and precursors. PHA synthase seems to be the most important enzyme involved in the process to obtain specific polymers, having a particular role in polymeric structure and conformation[21, 22]. PHA synthases are the main class of enzymes[22] that polymerize HA units in the cell within PHA-granules where they are located, which are generally attached to the cellular membrane[3, 23].

PHA synthases classes I and II are very similar, having one single subunit (PhaC) with a molecular weight of 65 kDa that polymerizes CoA thioesters of short chain length (scl-HAs) and medium chain length (mcl-HAs), such as 3-hydroxybutyrate (3-HB) and 3-hydroxyvalerate (3-HV)[24]. Class III is composed of two subunits (approximately 40 kDa each), PhaC and PhaE, and utilizes scl-HAs, ranging from three to five carbons in length. Classes IV and III are very similar, the difference being that class IV has another subunit, PhaR, that improves the enzyme stability in the PHA granule-structure[1, 6, 19].

Since PHA synthases are the major enzymes involved in polymerization, they are suitable for changes and mutation insertions to manipulate the way that the enzymes synthetize different polymers. The most common way to change the production is the addition of recombinant plasmids into strains with high production rates, such as Escherichia coli, from other organisms or from different species[25, 26]. Pseudomonas stutzeri and Pseudomonas putida[27], Cupriavidus necator [28] and Bacillus megaterium [29] are the preferred sources of PHA-related genes for applications involving genetic transformation.

b. USING BIOREFINERY AS A PLATFORM TO OBTAIN PHAS

Most of our chemical industry is based in fossil fuels and non-renewable resources, leading to a dangerous situation and imminent exhaustion of those sources of energy. In the late 90s, the term biorefinery started to be employed, and it initially involved looking for alternatives to replace common methods for non-food bioproducts and certain chemicals[30, 31]. The National Renewable Energy Laboratory defines a biorefinery as "a facility that integrates biomass conversion processes and equipment to produce fuels, power and chemicals from biomass;" thus, biorefinery takes the perspective of an integrated process that absorbs organic residues to transform them into value-added products and energy[32].

In a biorefinery, the whole process usually involves at least three steps: 1) selection of organic waste, 2) pretreatment and 3) insertion into the system to generate the desired products, with or without the generation of byproducts. In this context, two steps are crucial to an environmentally and economically friendly process, i.e., the pretreatment and the system to obtain the product[31, 33]. Thus, pretreatment can be carried out by three different approaches: physical, chemical or biological.

Physical pretreatment involves freezing, extrusion, microwave and heat processes to breakdown the main components of the organic waste. This process is environmentally safe but normally has high energy costs[34, 35].

Chemical pretreatments, such as oxidation, alkali or acid hydrolysis, organic solvents or ionic liquids, are some of the most effective single processes and are widely used on an industrial scale. However, there are several environmental aspects that need to be carefully monitored, since these pretreatments can generate toxic byproducts (e.g., chlorinated organic compounds) [31, 36].

Biological approaches to treat organic wastes seem to be the best option, considering the environmental and economic aspects. However, these pretreatment processes do not generate large yields of biomass ready for usage. They need to be improved for larger applications, such as the fuel and energy industry, green chemicals and new polymeric materials research[35, 37].

The costs to operate and implement a biorefinery are high, and they require skilled labor. These costs mean that the product obtained at the end of the process must have high market value or high demand, thus giving the project economic sustainability. With this premise, the idea is to modify the products obtained in the process, such as building blocks or chemicals, into new substances with specific markets. For example, the pharmaceutical industry is quite appropriate for the commercialization of this type of technology. In the case of HAs, there are numerous processes to convert the main building blocks such as 3HB, 5HV and 3HO (3-hydroxyoctanoate) into fine chemicals, drugs for Alzheimer's treatment and other noble applications.

III. TECHNIQUES TO MODIFY HAs INTO NEW BIOPRODUCTS

a. Chemical transformations, different applications and bioproducts from HAs

Since HAs can easily be produced from organic biomass (as described above) and purified, many approaches can be designed to modify those organic compounds into distinct bioproducts with noble applications. Reactions such as coupling, esterification, cyclization and substitution are the most common approaches to obtain new products from organic compounds, and HAs have great potential and diverse applications[10, 38]. Among the scl- and mcl-HAs, considering their polymeric forms, production rates and easy purification, HB, HV, HHx (hydroxyhexanoate) and HO stand out with respect to quantity and applicability[39].

The most common HA is HB, which is easily obtained from almost all microorganisms that accumulate PHAs. The methylation of 3-HB generates methyl-hydroxybutyrate, a molecule with a great potential in the medical field[40] that is used to combat Alzheimer's[41] and improve memory in mice[42]. This approach is an alternative to the high cost to obtain these molecules. Another use is to convert PHB to n-butanol[43], an important fuel with a high market value and applications in the chemical industry.

Recently, Dhamankar and collaborators[44], have created an efficient platform to obtain 3-hydroxybutyrolactone (3-HBL) from PHB through a biosynthetic pathway. Furthermore, the platform utilizes a recombinant E. coli to produce 3-HBL and 3,4-dihydroxybutyric acid (3,4-DHBA)[44] utilizing glucose as a carbon substrate. As described by Werpy and Petersen[45], 3-HBL and 3,4-DHBA have interesting market applications, including as precursors in the pharmaceutical industry and for the synthesis of important building blocks.

Another compound with medical applications is γ-hydroxybutyrate, a psychotropic drug that affects the central nervous system (CNS) and is known as "the date-rape drug"[46]. Commercially, γ-hydroxybutyrate is prepared as a sodium salt (sodium oxybate) and utilized to treat alcohol withdraw[47]. It is also consumed illegally by many young people because it causes a sense of euphoria, but it can cause severe toxicological effects and be fatal in high doses[48].

HV or hydroxyvalerate, another important HA, can be changed by adding a methanolic group at position 4 to generate 3-hydroxy-4-methylvalerate (3H-4MV) utilizing an approach developed by Saika and co-workers[49]. 3H-4MV has an interesting use as a flexibility-enhancing agent in polymers, changing their crystallinity[50]. HV is mostly found in a copolymer formation due to its capability to change crystallinity and cause changes in melting and glass transition temperatures[28, 51]. It is also used for medical applications[11, 52], including as a drug delivery system and in implants[40, 53].

Another HA with a variety of medical and clinical applications is hydroxyhexanoate (HHx), which can be used as a support material for tissue engineering[54], advanced drug delivery systems[55] and even as a polymeric material[56]. Since both are applicable in the medical field, HAs used for human implants or drug delivery systems need to pass a series of tests to avoid contamination by other compounds, especially endotoxins[9, 57]. In an early article, Kunz and Weimer[58] proposed the in vivo production of adipic acid by feeding Pseudomonas spp. 6-hydroxyhexanoate. Another utilization of HHx is in block copolymers[6] to change the aging properties of the main polymer, thus providing more stability and durability.

Along with HB in applicability, hydroxyoctanoate (HO) is a medium-chain length alkanoate that has many uses and sub products that can easily be obtained through modification of the main molecule. First, in the medical and pharmaceutical fields, HO can be used in a film form for pulmonary valve implants and drug-eluting stents[11], polyesters with antimicrobial activity[59, 60], drug-release systems[61], tissue engineering materials and other applications[40, 62, 63]. Second, graft polymers can be obtained, which adds a functional group in the polymer to generate a product with new characteristics[6]. Nano composites with graphene[13], that have a great added value, as well as chiral hydroxyacids[64] are options for more economical uses of HO.

IV. Final considerations and future prospects of HAs

PHAs have emerged as a new bio-based chemical platform for the green production of top-value products due to the diversity obtained from their byproducts and their applicability in different areas. Many microorganisms have been found to accumulate HAs, mainly in the form of 3-HB (PHB), but only a few have been exploited until now[6, 65]. Cheap substrates, such as xylose, palm oil, glycerol and malt waste, can easily be employed as raw materials to obtain HAs[1] with small modifications in the process and in the microorganisms utilized to carry out the conversion.

Looking at the polymers market, one of the most readily available organic molecules is HA, which appears to be the most promising building block, along with succinic acid and poly-lactic acid[65], but has lower costs compared to petroleum-derived compounds[66], though the production of petroleum-based plastics is still economically attractive for industry. Governmental incentives for R&D, tax exemptions or lower taxes and other fiscal obligations would support more initiatives for biopolymers and other products. Considering the long-term perspective, petroleum-based products such as plastics will be replaced gradually by green chemicals and renewable organic processes.

Other attractive sectors are the bio-medical and pharmaceutical industries, with promise in drug-delivery systems[52, 67], tissue engineering support materials[54, 68], scaffolding, and even drugs themselves[41, 42]. These applications need a pure polymer, without other contaminants, such as solvents[9] or toxins, such as LPS (lipopolysaccharides)[69] or other compounds that affect human health.

HA utilization in more diverse applications is still in the process of obtaining the raw materials needed for production. With advances in biorefinery technology and research on metabolic pathways that regulate HA synthesis, the process can reach optimal or sub-optimal ranges.

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