Polyhydroxyalkanoates (PHAs) produced by bacteria and its potential application to industrial level

Polihidroxialcanoatos (PHA) producidos por bacterias y su posible aplicación a nivel industrial

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Abstract

Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible plastics synthesized by a wide variety of microorganisms, which share similar characteristics with petrochemical plastics. The most recent studies focus on the pursuit of economic substrates and extraction strategies that allow reducing product costs and thus, can make inroads in a market widely disseminated where petrochemical plastics dominate. The aim of this review is to show the polyhydroxyalkanoates production as a resource and tool first hand and have great potential for use in applications ranging from the manufacture of disposable products commonly used to biomedical and pharmaceutical products high added value.

Key Words: bioplastic; biopolymers; polyhydroxyalkanoates; microorganisms; biosynthesis.

Introduction

Plastic are polymers which have given various uses in the industry, from the pharmaceutical, to the food and transportation, among other, becoming in the base of most of the consumer products (Grados *et al*., 2008) and replacing the glass packages due to its properties such as elasticity and flexibility (Arcos, 2007), besides of its easy workability and high chemical resistance (Castillo, 2008). The conventional polymer, are mainly produces as a derivation of the petrochemical industry and last in the environment for long periods of time, due to its high weight and molecular composition, which makes the material recalcitrant and resistant to the degrading action of microorganisms (Khanna & Srivastava, 2005; González *et al*., 2013), they are not biodegradable and they bioaccumulate in the ecosystems, producing big quantities of toxic wastes that notoriously affect the environment (Wendlandt *et al*., 2005)

On their part, bioplastics are a kind of material that in the decades, have been used to replace the plastics deriving from petroleum and in contrast with these, they can be produces from renewable energy sources (Rozsa *et al.*, 2004; Arroyave *et al.*, 2013). The European Associaton of Bioplastics classification them as: 1) Synthesized plastics developed from renewable sources and 2) Biodegradable polymers that accomplish with all the criteria of the scientific recognized laws of biodegradability and according to the European standard EN 13432 (Industry Experts, 2012) such as Polyhydroxyalkanoates (PHA).

It has been explored and deepened in different processes to recycle or dispose of petrochemical source plastic of the environment, the socio-economical tendency towards the sustainable development have fuelled the research of this generation of polyhydroxyalkanoates (Verlinden *et al.*, 2007), that offer a better bio-match, causing minor impacts in the ecosystems. No doubt that the challenge of this technology of clean production is based in obtaining bioplastics with similar properties to the plastics based on petrochemic in the market with a similar or lesser cost (Rivera, 2009).

Polyhydroxyalkanoates

The biopolymers or Polyhydroxyalkanoates (PHA) are considered strong candidates for the replacement of petrochemical origin polymers, since being synthesized by microorganisms departing from low or null economical substrates and in general renewable resources, they have physical features similar to the plastics derived from oil, such as polypropylene and polythene (González *et al.*, 2013; Lee, 1996; Sudesh *et al.*, 2000; Serrano, 2010), but have the possibility to be degraded by carbon dioxide and water in aerobic conditions or methane in anaerobic conditions (Du *et al.*, 2001), In such diverse habitats like the soil, sea, stagnant waters and waste water.

These, are polymer of hydroxyalkanoical acids that are some microorganisms that intracelularlly accumulate hydroxyalkanoical acids as a reserve material to be later used as a source of acids and energy. The polymerization of the hydroxyalkanoical acids, due to the action of intra-cellular enzymes have a place through condensation of the one monomer carboxyl group (hydroxyalkanoical acid),with the hydroxil group of the next, forming an ester link; from there on, they will be called biopolyesters (Khanna & Srivastava 2005) (Figure 1).

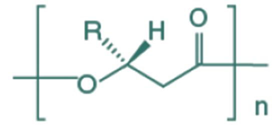

Figure 1. Chemical structure of the PHA
Source: (taken from Andler & Díaz, 2013)

The value of n will depend of the R group and the microorganism in which the biopolymer produces, varying between 100 and 30000 (Lee, 1996a). In the Table 1, it is indicated that the most common sustitutions are the radical group and the name that the polymer receives (Andler & Diaz, 2013).

Table 1. Sustitutions in the radical change and some of the best known PHAs

R Group	Name of the Polymer	Abreviatión
CH_3	poli(3-hydroxybutyratr)	PHB
CH_2CH_3	poli(3-hydroxyvalerate)	PHV
$CH_2CH_2CH_3$	poli(3-hydroxyhexanoate)	PHHx

Source: Taken from (Andler & Diaz, 2013)

Despite all of the environmental advantages of the PHAs in front of the petrochemical plastics, the main problems is that it confronts the production of this polymer is its high cost (Andler & Díaz, 2013), why action is being taken to make them more accessible to the population, between the ones that figure a better fermentation and extraction process.

Authors like Salehizadeh & Van Loosdrecht 2004, point out the research abput PHA that has given place to crops with a polymer concentration of more thatn 80 g/L and with a productivity of more than 2 g/L/h, using systems by fed batch or continuous, that potentially could reduce 50000 tons per year, this way marking use and potential exploitation of this polymer (Salehizadeh & Van Loosdrecht, 2004)

On other part, for authors such as Dai and contributors (2007), the PHA production through fermentative processes with microorganisms, can be performed with a microorganism (pure crop) or a consort of microorganisms (mixed crops); however the economic production is going to depend not only in raw material costs of the coil but in the conditions of asepsis of crop (monocrops) that are the most advanced technology or the mixed crops production (Dai *et al.*, 2007).

The PHA production using mixed crops, offer an advantage in front of pure crops due to this kind of fermentations the sterility conditions are not required. (Arcos, 2007). In any other case, it is required to stablish the operational parameters involved in the fermentative process, being the most incidence the temperature, the pH and fermentation time (Kulpreecha *et al.,* 2009). Although, until now the challenge keeps bieng the bioprocess of developing with high efficiency of PHA conversion to the lowest cost possible (Andler y Díaz, 2013).

PHA classifications

The Polyhydroxyalkanoates are classified according to the nature of the monomer units, if the polymer is formed by only one type of units, it is denominated as homopolymer (Anderson & Dawes, 1990); at the same time, if it is integrated by monomer of different length of carbon atoms in the same pellet, then it refers to copopolymer (Zhao y Chen, 2007).

There are three known types of PHA according to the length of the lateral chain PHA scl (short chain length), PHA mcl (medium chain length)and PHA lcl (long chain length) where the lateral alkyl chain varies from 1 to 14 carbons (Cardona, 2012). The principal charactristics of the PHA scl is that they are very stiff and fragile, meanwhile the mcl are more elastic but endowed with less mechanical force (Ortiz, 2009). It must be mentioned that the reason why a PHA is formed when a polymer of broken or médium chain is directly related with the enzyme in charge of the synthesis (sintasa) since this is the specific substrate and can act upon monomers with different number of carbon atoms (Anderson & Dawes, 1990); this way the chemical composition of the PHA will depend in the used substrate, the enzyme of the PHA-sintasa and the involved metabolic ruled (Lemos *et al*., 1998).

Inside the PHA, the poli(3-hydroyibutyrate) or PHB is one of the most studies and is synthesized by different bacteria (Gumel *et al.,* 2013). Posses very similar characteristics to polypropylene (PP) that is one of the conventional plastics (of the petrochemical industry) of major use. In the Table 2 a comparison between the two polymers.

Table 2. Comporting physical properties (PP)

Parámetro	PHB	PP
Fusion temperture(ºC)	177	176
Glass transition temperature (ºC)	2	-10
Cristalinity (%)	60	50-70
Tension strenght (MPa)	43	38
Extension until break (%)	5	400

Source: (Jendrossek & Handrick, 2002).

Synthesis and PHA microbial accumulation

If well, it has been detected PHA in different bacterian species, the percentage of accumulation in them es very low, which so are rejected in upon dificulty or impossibility to industrialize the market (Ieczak *et al.,* 2013; Andler & Díaz, 2013); currently at least 75 different bacteria genders produce PHA, as negative Gram, as positive Gram, that acumulates it im the citoplasma under certain regios and determined crop conditions (Scherer *et al.,* 1999; Bello *et al.*, 2009 y Suwannasing *et al.,* 2012).

It is meant to point out the production of granules of PHASm is induced under a series of unfavorable conditions for the bacterian growth, with which these polymers are produced and accumulated intracelularlly as an energy storage when the bacterial cells are found in said conditions of stress that generally can be nutritional as the limitation of phosphorus, nitrogen, oxygen or in a not optima pH and a carbon. It has been reported that in one bacteria it can reach up to a 90% of its dry weight in PHA molecules (Madison & Huisman, 1999).

The PHA are accumulated as liquid polymers, mobiles and amorphous in the form of granules located in the micoorganism's cytoplasm, surrounded in a monolayer of phospholypids that have enzymes, polymerases and despolymerases (Figure 2) (Ábalos *et al.,* 2003; Gómez, 2013). These bacteria synthesize the PHA as an storage compound in carbon and energy sources (Lenz & Marchessault, 2005; Ojumu, *et al*., 2004) and probably carry out other functions in the bacterian cell. Hence, constituting a group of biodegradables materials of high biotechnologic potential (Arcos, 2007).

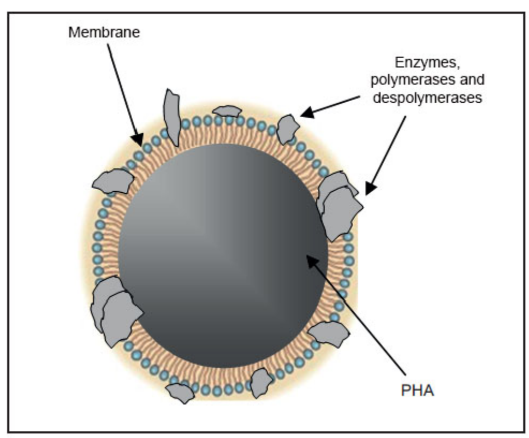

Figure 2. Scheme of PH granule accumulated intracellularlly
Source: (Sudesh *et al.,* 2000).

Previous researchs have been donde abput the processes of production and accumulation of the PHA indicate that a number of granules per cell are defined in the first stages of the process and the production of the polymer ceases when the accumulation content reaches between 80-90% of the celular weight in dry nase of the bacteria (Catone, 2013). This phenomenon has taken to the conclusión that physical restrictions exist that don't allow the cell to store more polymer, despite having the avaliability of the substratum and the activity of the polymerase enzyme (Wang & Lee, 1997). These conclusión are observed under the microscope as spherical granules of different sizes (Braunegg *et al.*, 1998).

Taking in account that the PHA is an storage compound departing from an excess of carbon, it is natural that the microorganisms count with an enzyme to degrade it and thus recovering the stored carbon when in there is lack of this in the surrounding enviroment, this way the PHA can serve as a carbon or energy source for the microorganisms during a fasting period or nutritional (Punrattanasin, 2001; Arcos, 2007). In bacteria as the *Ralstonia eutropha*, it has been observed that the degradation rate of the stored polymer is ten times slower tan the synthesize rate. However the mechanism of PHA degradation has not been researched yet as extense as the synthesize (Doi *et al.*, 1992).

Nevertheless, there are also bacteria that present a polymer production associated with growth, although in this case it is less frequent (Lee, 1996a). In the Table 3, are shown the main nutrients whose limitation give place to a PHA synthesize in different bacteria.

Finally, the choice of the microorganism for the biopolymer's industrial production will vary depending in the factors like the celullar capability to use non-expensive carbon sources, the growth speed, the biopolymer's synthesize speed, the quality and quantity of the PHA and the recovery processes expenses (Lee y Choi, 2001). At industrial level strains like the *Ralstonia eutropha, Alcaligenes latus, Azotobacter vinelandii, Pseudomonas oleovorans, Paracccus denitrificans, Protomonas extorquens & E. coli* are used (Lee, 1996b; Fernández *et al.*, 2005; Arroyave *et al.*, 2013).

Mundially, the most studied microorganism is the *R. eutropha* bacteria due to its capability to store great quantities of PHA, specifically the poli-b-hidroxybutirate type (PHB), in a percentage close to the 80% of the bacteria's dry weight. This productiong and storing, can be carried out by a simple enviroment from economic carbon sources such as glucose, fructose and oil industry waste, agroindustrial, etc (Lee, 1996b; Priyadarshi *et al.*, 2014).

Table 3. Main nutrients whose limitation give place to the production of PHAs in different microorganisms

Microorganism	Limitating nutrient
Alcaligenes latus *Pseudomonas oleovorans* *Pseudomonas cepacia* *Ralstonia eutropha* *Rhodobacter sphaeroides*	Nitrogen
Pseudomonas sp. K.	Magnesium
Azotobacter vinelandii *Azotobacter beijerinckii* *Rhizobium* ORS571	Oxygen
Rhodospirillum rubrum *Rhodobacter sphaeroides* *Caulobacter crescentus* *Pseudomonas oleovorans*	Phosphorus

Source: (Babel & Steinbüchel, 2001).

It is worth to mention that in the last years the number of articles and researches that are developed scoped in production, advantages and disadvantages in the collecting of PHA are more frequent and many more. In them, many physical-chemical factors have been evaluated and tested with the goal of knowing and stablishing those that represent more incidence in the production of these biopolymers. For example, Kasemsap & Wantawin (2006) in Thailand researcged the productivity and the intracelular content of PHA departing from actívated sludges from waste waters that were used as carbon sources from a mix of native microorganisms of this substratum to test the effect of the pH over the production capability of the biopolymer. These researchers found that the obtained performances by mixed crops, can be compared with the ones of the pure sludges if the process had an anaerobic stage and a pH increase, because the form of the cell needs less energy, and seizes the delivered energy from the polyphosphate degradation in anaerobic conditions (Castro *et al.*, 2011), demonstrating once more that the PHA production from activated sludges is an innovating solution that reduces notoriously the process costs, is enviromental friendly and can manage more flexible operations. (Salazar, 2010).

It must be highlighted, that different substrates have been used along the last years, between them, residues from agricultura such as the rice husk and the cornstach, demonstrating that these substrates, not only reduce the costs of the material, but they increase the celullar concentration and PHA accumulation (Salazar, 2010).

From different essays in Huang laboratory level and partners in the 2006 tested two types of substrates: rice husk and cornstach and as sludge *Haloferax mediterrane* in the capability of PHA production. They found ound that this sludge can not grow from rice husk in comparison to the surprising results that were obtained when using the cornstach as substrate, where the concentration of PHA reached the 77,8 g/l and a content of 55,6% PHA of dry cellular weight, representing two times more celular concentration and three times the PHA concentration if only cornstach is used. These results, make this process potentially viable in great scale if the same fed-batch is continued, and under the same parameters used in a laboratory level (Huang *et al.,* 2006).

Industrial application

Although they are not very marketed yet nor presents a high worldwide demand, some industrial appliactions for the PHA have been already described, among the the fabrication of thin covering coatings; linking agents in water-based ink formulations; as source of chiralic monomers for the synthesize of active compounds as support of tissue engineering and temporary medical implants. (Vergara, 2012).

Due to the fact that it contains a large polimeralization grade and a cristalinity grade in the range of 60 to 80%, are optimally active (because they present a chiral carbon), isotactic (that is to say, they are formed by stereo chemically regular repeated units and insoluble in water. Making them highly competitive with the propylene and other plastics derived from petroleum (Reddy *et al*., 2003; Madison & Huisman, 1999).

These are the properties, that allow the PHA to be used in molding processes (González *et al.,* 2013). Nonetheless, the most know application of these short-chained biopolymers is the manufacturing of disposable bottles, bags and other disposable products such as diapers, napkins, razors, glasses and cutlery (Anderson & Dawes 1990).

Besides, they can be used in the cover of fibrous materials like paper or cardboard from the shape of aqueous latex. Thus, due to its high water resistance, this cover protects the paper or carboard against the deterioration by humidity (González *et al.,* 2013).

Colombia, just like some Southamerican countries, don't show meaningful advances in the orientation and production of PHA; in the last years it has been researched advances in laboratory scale. Inbetween them, is the reported case in 2008 in the Colombian Magazine of Biotechnology, about the Universidad Nacional de Colombia, where an innovating technology that allowed to free toxins from the sludge *Bacillus thuringiensis* troughout the use of PHA granules was published. This techonology would fin dan importan environmental solution, because the produced toxin by this microorganism would be immobilized inside the PHA granule, that forwardly would be used as a biological plaguecontrol, this way when immobilizing the toxin inside the granule, there is the possibility to modify parameters such as the size of the granule, thus incrementing the content of said toxin inside it. (Rosas, 2008).

In the agriculture, the application of the short-chain PHA is developed in biodegradable pots, irrigation tubes and matrixes for the controlled liberation of growing factors, pesticides and herbicides. One advantage of this field of application is that a high grade of purification of the polymer is not required, which can ease the process of extraction and making it cheaper (Babel & Steinbüchel 2001 quoted by Gonzalez *et al*., 2013).

It could be observed that the PHA prodcution began to be important to our environment and even though it industrial production is not that viable due to the large estimated times to imporve the processes, it is highlighted that these bioploymers start to get known as a rasing techonology and of potential use to satisfy the different need of each country in the future.

One of the stratetigues to make the competitive the production of biopolymers in an environmental, technological and even economical point of view, would be the seizing of agroindustrial residues in the PHA production; thus two important aspects would be worked in different sectores: 1) In the agroindustrial sector, creating an alternative to the seazing of a subproduct, given in many ocations, high environmental have been generated in their final disposal or in the generation of a high economic invest for its treatment and 2) In the application of environmental friendly technologies, from the industry of biopolymers, specifically the PHB, this because of the reduced production costs and would give and added value to a polluting raw material (Naranjo, 2010).

In Colombia and specifically in the department of Valle del Cauca where the agricultural activity has an important participation in the nacional and regional economy and at the same time generates big quantities of solid and liquid wastes (some of them with contaminating and recalcitrating characteristics), the exploration of different alternatives to guide and develop an enviromental manage of the agroindustral wastes is a priority (Sánchez *et al.*, 2012), becoming them a key point in the biotechnological seizing as is the production of PHA.

This way, this compilation pretends to induce and/or guide works and studies, that can cover the different problematics of transformation and optimization of the implicit variables in the process of production and accumulation of the PHA and that they propose solution alternatives and contributions that slowly direct the way to the exploitation of these residues for this purpose.

Conclusions

The Polyhydroxyalkanoates are versatile biopolymers with diverse applications in industries suchas the: pharmaceutical, biomedicine, foods, packaging, among others. The PHA are biodegradables and can be produced in a sustainable and eco-friendly way, utilizing renewable raw materials or industrial waste.

Although, the PHA are recognized as candidates to produce biodegradable plastics, they still have limitation for their production in a high scale, its high price in comparison to the conventional plastics, for this is necessary to look for an eco-friendly alternative and economically justified to produce it.

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