

Polyhydroxyalkanoate Research in South America in the Last Six Years: A Review

Parra Boris¹ and Fernandez-Bunster Guillermo^{2*}

¹Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile

²Facultad de Medicina, Universidad de Valparaíso, Camino la Troya Esquina El Convento, San Felipe, Chile

*Corresponding Author: Guillermo Fernandez-Bunster, Facultad de Medicina, Universidad de Valparaíso, Camino la Troya Esquina El Convento, San Felipe, Chile.

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Abstract

This review aims to name and describe the research in South America on polyhydroxyalkanoates (PHAs), one of the most studied and analyzed biopolymers obtained from biological sources. In the last five years, many publications on this topic have surged from several South American countries. Sadly, a great number of these publications appear in databases that are not commonly used by non-South American researchers, such as SciELO and national journals. Several groups have worked in the improvement of the capacities of PHAs, in terms of testing cheap carbon sources, improving metabolic pathways in the native strain or by transferring the synthesis genes to other strains, evaluation of the polymer properties depending on the use, among others, but the situation of unknown databases may produce an unexpected difficulty to find common research to achieve fruitful cooperation between research groups. Ultimately, the main objective of this manuscript is to solve this problem by informing other researchers about PHA work in South America.

Keywords: Bioplastics; Biopolymers; Polyhydroxyalkanoates; South America; Biodegradable Polymers

Abbreviations

PHA: Polyhydroxyalkanoate; PHB: Polyhydroxybutyrate; PHBHV: Polyhydroxybutyrate-Co-Hydroxyvalerate; scl: Short-Chain Length; mcl: Medium-Chain Length

Introduction

One of the main environmental pollutants is petroleum-derived plastic, such as polyethylene (PE) and polypropylene (PP), which are part of our everyday life in forms of packaging, bottles and containers, among others. These types of plastics remain relatively unchanged over time. As a replacement, natural biodegradable plastics can be used, such as polyhydroxyalkanoates (PHA - Figure 1), polyesters that can be accumulated into the bacterial cytoplasm as granules or inclusion bodies. In general, to stimulate its biosynthesis, carbon must be in higher proportions than other nutrients, such as nitrogen and phosphorus [1]. Polyhydroxybutyrate (PHB) is the most common example of PHAs, but the monomer structure of these polymers is highly dependent on the bacterial strain and the fermentation properties.

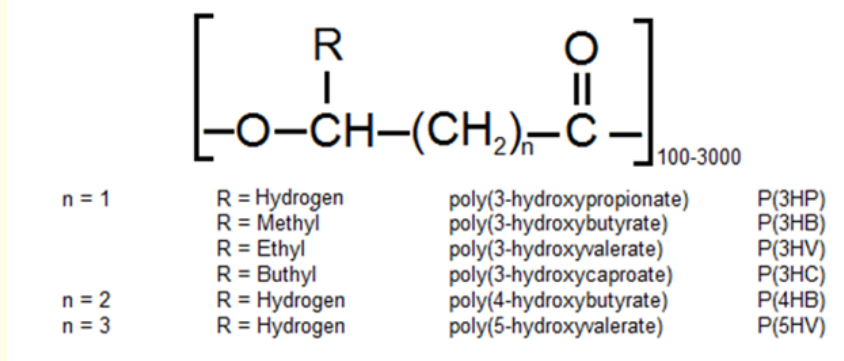


Figure 1: PHAs general structure.

Despite the biodegradability and physicochemical properties of PHAs, their production costs are higher than petroleum-based plastics. Several South American research groups have worked in the improvement of the capacities of these biopolymers, in terms of testing cheap carbon sources, improving metabolic pathways in the native strain or by transferring the synthesis genes to other strains, evaluation of the polymer properties depending on the use, among others [2]. South America is prolific in polyhydroxyalkanoate research and this review aims to describe investigations made on the continent to approach several research groups, to enhance networking and joint work between South-American and worldwide groups. The sections were chronologically arranged and divided as 1) PHA accumulation based on different carbon sources and/or growth conditions; 2) PHA metabolic pathways analysis and modifications; 3) Draft genome sequencing, describing PHA-related genes and bacterial strain-description; and 4) PHA blends in form of nanoparticles or membranes for biomedical applications.

Materials and Methods

Several databases were used to search for research based on PHAs, namely SciELO (<https://scielo.conicyt.cl/>), NCBI (<https://www.ncbi.nlm.nih.gov/>), Google Scholar (<https://scholar.google.com/>) and ResearchGate (<https://www.researchgate.net/>), using terms related to polyhydroxyalkanoates, such as "PHAs", "Polyhydroxybutyrate", "Biodegradable polymers", among others, and South American countries as sub-criteria. The selection criteria were: The research must be made in a South American country, or a South American researcher participated as an author, as well to be in Spanish, English or Portuguese, as well from 2014 to 2019.

Polyhydroxyalkanoates research in South America

PHA accumulation based on different carbon sources and/or growth conditions

A great part of the South American research on PHAs is based on the evaluation of novel carbon sources in different bacterial strains. Sometimes, the PHA accumulation characteristic of certain bacterial strains can be used jointly with other metabolic activities, such as the removal of contaminants, as can be seen from 2005. Hydrocarbon-contaminated sites are stressful environments for the development of microorganisms, due to the presence of toxic compounds such as benzene, toluene, and xylene (BTX). Selection of bacteria with the capability to tolerate and degrade monoaromatic compounds, to synthesize biosurfactants and/or to accumulate biopolymers that enhance stress tolerance could be a good approach to find a suitable bioaugmentation agent.

In terms of the amount of research, 2014 was very productive: A study about PHA production *in Bacillus sp.* using different carbon sources, such as glycerol, fructose, glucose, lactose, and milk serum. After a comparison of bacterial growth and PHA accumulation parameters, 5 gL⁻¹ glycerol was the most promising, but no PHA quantification was performed. Also, the dependence of the yeast extract and glycerol in bacterial growth was evaluated, linked to PHA production, showing that PHA production is directly dependent of yeast extract, but independent of the glycerol content [3]. Other group used crude glycerine, a by-product of the biodiesel industry, in a fermentation process using the bacterial strain *Cupriavidus necator* IPT 026, 027 and 028, obtaining up to 2.82 gL⁻¹ of PHA in 72h of growth at 35°C. The bioaccumulated PHA had three different methyl esters of PHAs in their composition, and as other industrial by-products, crude glycerine can be used as a cheap substrate to accumulate PHA [4]. Also, other strain of *C. necator* (DSM 545) used waste wine, a by-product of sugar beet fermentation, as a food source: Tests demonstrated bacterial strain growth using diluted waste wine as an energy source, and when essential substrates were added, together with a source of nitrogen and glucose, the strain produced PHB [5].

In *Pseudomonas oleovorans*, sugary cassava extracts supplemented with andiroba oil were tested as energy and carbon source, respectively, to accumulate mcl-PHA. Andiroba oil was added at different growth stages, resulting in an accumulation of 3-hydroxy-decanoate and dodecanoate units, PHB, 3-hydroxy-hexanoate and 3-hydroxy-octanoate monomers. PHA was accumulated mainly at the stationary phase, when oxygen was limited but with enough nitrogen concentration to keep cells growing. Results were conclusive enough to show the potential of cassava extract for PHA production [6]. A Chilean group proposed to use raw glycerol as a carbon source in different strains of *P. putida*. Best results were obtained in *P. putida* KT2440 synthesizing more than 34% of its cell dry weight as mcl-PHA under

nitrogen-limiting conditions. KO of the PHA depolymerase gene *phaZ* enhanced the production up to 47% PHA (%w/w), a low value considering other engineered microbes, which was probably triggered by the high production of citrate, a side-product of the metabolic pathway. Overall, the research shows how important it is to choose the appropriate microorganism for PHA accumulation from waste materials, and a simple flux metabolism analysis could help to avoid undesired compounds that diminish the availability of precursors for biopolymer synthesis [7].

Another research on *P. putida* investigated the enzymes involved in mcl-PHA precursor formation. The research was based on former studies focused on the inactivation of the *gcd* gene (glucose dehydrogenase gene), which produces side products such as gluconate. The inactivation of that part of the metabolic pathway resulted in a two-fold increase of mcl-PHA. An *in-silico* model revealed potential genetic targets identified by flux analysis, such as the overexpression of a pyruvate dehydrogenase subunit *AcoA* resulted in a 33% more PHA production, while together with the *gcd* mutant, the accumulation was enhanced up to 121%, together with the induction of the expression of glucose 6-phosphate dehydrogenase and pyruvate dehydrogenase genes. Finally, a relation between NADPH and PHA synthesis was described, in which a high biopolymer concentration was observed at low NADPH levels. They obtained biopolymers ranging from C6 (3-hydroxyhexanoate), C8 (3-hydroxyoctanoate), C10 (3-hydroxydecanoate), C12 (3-hydroxydodecanoate) and C14 (3-hydroxytetradecanoate) [8].

Also, in *P. putida*, different fed-batch strategies were tested to increase PHA production, using glucose as a carbon source. By metabolic engineering, they modified the genes glucose dehydrogenase *gcd* and the 6-phosphogluconolactonase *pgl*. Their strategy consisted of an initial phase of biomass accumulation by exponential feeding and then, at the PHA accumulation stage, three different induction techniques were tested under N limitation: Constant-fed, pulse-fed and DO-stat (Dissolved Oxygen Stat Feeding Strategy). Results showed that substrate pulse feeding has better values than constant feeding in terms of PHA accumulation. DO-Stat obtained the highest values for the strain at the Δgcd mutant, with a 67% PHA content and specific PHA productivity of $0.83 \text{ gL}^{-1}\text{h}^{-1}$, highlighting how crucial is feeding strategy in biopolymer accumulation. The engineered *Pseudomonas* strain was able to produce 100% more mcl-PHA than the wild-type using glucose as the sole carbon source. Additionally, PHA synthesis stimulates the production of compounds that affect the surface tension but not the production of emulsifiers, as well as the PHA accumulation affected cellular affinity to xylene [9].

The following year, the macromolecular composition and PHB accumulation of activated sludge was tested with the synthetic sewage water. Despite changing the conditions of oxygen, organic load rate, and temperature, the activated sludge PHB storage was very low for all conditions tested [10]. Carob pulp and figue juice were tested as a novel carbon source: The strain cultured with carob pulp showed an excellent efficiency when compared to sugar cane molasses [11], similarly as stated by other research groups.

In 2015, studies were performed on halotolerant bacteria, *i.e.* able to grow in salt, and its related bio-products: The bacterial strains were obtained from the Sao Paulo Zoo composting process, processing up to 4 tons/day of residues. The organic residues contained plant matter from the rainforest, animal manure, and mud from water treatment. From the bacterial screening, eight halotolerant strains were found and classified by using 16S rRNA gene sequence analysis, being identified as members of the genera *Staphylococcus*, *Bacillus* and *Brevibacterium*. From all the strains, *Brevibacterium avium* showed enzymatic activities (cellulase and amylase) and produced exopolysaccharides and polyhydroxyalkanoates, indicating the industrial potential of microorganisms obtained from the composting process [12]. Other carbon source tested was sugar hydrolysate from Brewer's spent grain in *P. aeruginosa*. For this purpose, sugar was hydrolyzed using acid, followed by aerobic fermentation of the bacterial strain using mixtures between the hydrolysate and nutrient media in batch cultures. Biomass and PHA content were evaluated under different conditions and the best biomass enhancement results were obtained at 30°C with 20 g/L of sugar concentration. PHA accumulation was better accumulated using only 10 g/L of sugar, with a yield of PHA/biomass of 0.005 g/g. The low PHA yield was explained by the presence of hydroxylated inhibitors [13].

In *R. eutropha*, cassava flour was tested with different C: N ratios, demonstrating that a proportion of C: N 20:1 was the most favorable ratio for PHA accumulation [14]. For this part, bacterial growth and PHA production in *Burkholderia sacchari* LFM 101, using glucose,

glycerol and/or sucrose as a sole carbon source at 30°C and 35°C was tested [15]. These authors focused primarily on the factors that could influence bacterial growth, such as temperature effect on growth rates, and growth yields with different carbon sources. *B. sacchari* cultured with glucose at 35°C presented both higher productivity and polymer yield in dried cell mass, while no difference in growth rates could be noticed in sucrose. On the other hand, glycerol did not show good results at any of the different growth conditions, due mainly to the incapability of glycerol to be used as an energy supplier.

Continuing with their research on *H. seropedicae*, PHA synthesis was evaluated in a genetically modified strain to use lactose (*lac+*) by using whey permeate, a dairy industry by-product, as a carbon source. They obtained a PHB content of 38.5% for cells grown with 30.0 gL⁻¹ lactose in whey permeate. Afterward, a feeding experiment was tested by using shaking flask cultures split into three subcultures after 54h of growth using whey permeate at 10.0 gL⁻¹ lactose and 10mM NH₄Cl as final concentrations, obtaining a PHB content of 58% [16]. The same year, starch from potato peels (*Solanum tuberosum* L) was used as a carbon source for PHA production on native halophilic bacteria isolated from water samples, demonstrating that the potato peels can be used as C source for PHA production by native halophilic bacteria [17]. Mutations were induced by UV on the PHA-natural producer *Pandoraea* sp. MA03 to obtain mutants unable to grow on propionic acid (PA), but still able to produce [P(3HB-co-3HV)] from glycerol and PA at high 3HV yields. The mutant prp25 showed a high conversion rate of PA into 3HV units of 0.78 g g⁻¹ and further experiments with using fed-batch fermentations were performed using crude glycerol and PA, reaching a 3HV yield of 1.16 g g⁻¹, showing that the mutant can be used as an alternative to minimize costs and support biopolymer production from biodiesel by-products [18]. In 2018, PHB production was evaluated in *C. necator*, with the particularity of using a Central Composite Rotational Design (CCRD) for growth, showing that an increase in sugar concentration and temperature increased PHB accumulation [19].

PHA metabolic pathways analysis and modifications

Phasins have a structural role as part of the PHA granule cover, but also different functions can also be associated with this protein family, as reported in 2014. PhaPAz, a phasin from *Azotobacter* sp. FA-8 expressed in *E. coli* strains, has chaperone-like functions both *in vitro* and *in vivo*, preventing *in vitro* thermal aggregation of a citrate synthase protein and facilitating the refolding process of this enzyme after chemical denaturation. By microscopy techniques, they demonstrated that PhaP colocalizes with inclusion bodies of PD, a protein that aggregates when overexpressed, and produces a reduction in the number of inclusion bodies when coexpressed with PhaP [20], followed by structural characterization of PhaPAz, reporting that the protein lacks clear hydrophobic domains, despite their lipid granule binding capacity. The secondary structure of this protein, a tetramer mainly constituted by α -helices and disordered regions, has a remarkable capacity to change according to the environment. This structural feature was also found in other phasins and may be related to their functional diversity [21].

PHB synthesis regulation in *Bradyrhizobium diazoefficiens* was studied in 2016: Mutation of *phaR* impaired PHB accumulation, while a *phaP1/phaP4* double mutant produced more PHB than the wild-type, accumulated in a single and larger cytoplasmic granule than common parameters. Moreover, PhaR negatively regulated the expression of *phaP*, *phaA*, *phaC* and *fixK2* (transcription regulator). Besides, *phaR* mutants accumulated more extracellular polysaccharide, promoted higher plant shoot dry weight and competitiveness for nodulation than the wildtype, by contrast to *phaC1* mutant strains, defective in PHB synthesis. The research group suggested that PhaR not only regulates PHB granules formation but also acts as a global regulator of excess carbon allocation and symbiosis [22]. On *P. putida* KT2440, studies on the transcriptome and metabolic fluxes (fluxome) of the glycerol metabolism were performed, which is used to synthesize mcl-PHA, aiming to obtain a systematic understanding of the underlying metabolic and regulatory network of the glycerol metabolic pathway. Their research showed the relation of different gene expression and metabolic fluxes under PHA and noPHA producing conditions, using glycerol as carbon substrate on *P. putida*, describing that glycerolgrown *P. putida* KT2440 has a maintenance energy requirement about sixteen times lower than that of other bacteria, providing a great advantage to use this substrate commercially, aiming to decrease the production costs of the biopolymer [23].

Interesting research focused on cell lysis was published in 2017, considered crucial for the microbial production of products derived from microorganisms. They developed a novel programmable lysis system based on the heterologous expression of lysozyme and tested

on *E. coli* and *P. putida* KT2440. As stated in the research paper, they created an effective autolysis system for cell disruption, able to be activated at different growth stages during the batch fermentation process, recovering up to 75% of the total synthesized polyhydroxyalkanoate at the end of the fermentation period. Also, the proposed lytic system is prone to optimization by testing other promoters that do not affect total biomass and PHA formation [24]. On phasins, PhaP (from the soil bacterium *Azotobacter sp. strain FA8*) protects recombinant *E. coli* against several kinds of stress. i.e. PhaP enhances growth and PHB synthesis in recombinant strains reducing the formation of inclusion bodies during the overproduction of proteins and increase tolerance to several bioproducts. Additionally, PhaP enhanced bacterial fitness in the presence of biofuels, such as ethanol and butanol, and to other chemicals, such as 1,3-propanediol. The effect of PhaP was also studied in a groELS mutant strain, in which both GroELS and PhaP were observed to exert a beneficial effect, depending on the chemical tested. The potential of PhaP and GroEL to enhance the accumulation of ethanol or 1,3-propanediol was analyzed in recombinant *E. coli*. Their results demonstrated that overexpression of either *groEL* or *phaP* increased bacterial growth, reflected in a higher final biomass and product titer compared to the control. This novel application to the already multifaceted phasin protein group, suggests that the expression of these proteins or other chaperones can be used to improve the synthesis of diverse biotechnologically relevant chemicals from renewable carbon sources [25].

Finally, a mutant strain from *H. seropedicae* Z69 used propionic acid as a carbon source to generate 3HV. They successfully eliminated the 2-methylcitrate synthase *prpC* gene of the 2-methylcitrate cycle for propionate catabolism, achieving 14.1 mol% of 3HV; greater than that of strain Z69 (2.89 mol%) [26].

Draft genome sequencing, describing PHA-related genes and bacterial strain-description

Whole-genome sequencing, as well as gene identification by its sequence, has been a cornerstone on the discovery of novel genes related to polyhydroxyalkanoate production, as well as a helpful tool to characterize bacterial strains producer of different types of PHA.

In 2014, just three research papers could be found: The description of another PHB cluster *phbFPX* in the genome of *P. extremaustralis*, with high similarity to genes belonging to Burkholderiales and a cluster *phaC1ZC2D*, coding putatively for a mcl-PHA synthase, indicating that the ability of *P. extremaustralis* to produce high amounts of PHB could be explained by the different expression levels of the genes encoding the scl and mcl PHA synthases [27]. Further analysis of the genome of the same bacterial strain, detected genes with probable foreign origins such as those coding for acetate kinase, osmotic resistance, and colanic acid biosynthesis. These findings suggest that, in *P. extremaustralis*, the horizontal transfer events and/or gene redundancy could play a key role in survival under unfavorable conditions [28].

In 2016, the strain JA12 was isolated from an acid mine drainage water and characterized: The strain was described as a member of the genus *Ferrovum* (only another known member was *Ferrovum myxofaciens* P3G). *In-silico* analysis showed genes encoding for acetyl-CoA acetyltransferase and reductases and also a PHA-synthase was found, presumably enabling the strain to accumulate PHB. It also contained two copies of a gene encoding for a PHA depolymerase, involved in PHB catalysis [29]. Also, this year, in a Brazilian-Peruvian joint research, the draft genome sequencing of *Halomonas sp. HG01* was published, isolated from a Peruvian salt mine. The strain was described as an aerobic halophilic and heterotrophic bacterium that accumulates PHB and PHBHV using different carbon sources [30]. Another group (Silva, *et al.*) evaluated PHA accumulation in twenty-four bacterial strains isolated from an Atlantic forest soil located in Maceio and from an agro-industrial sludge in Caruripe (Brazil). Four of them contained the *phaC* gene, corresponding to *Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Klebsiella oxytoca* and *Bacillus pumilus* [31].

Finally, in 2018, a Peruvian research group successfully isolated PHA-producer bacterial strains from soil and water from salty Peruvian lagoons, obtaining as the highest yield of 73% of the cellular mass in PHA [32].

PHA blends in the form of nanoparticles or membranes for biomedical applications

Brazil and Chile stand out in the nanotechnology area, by the evaluation and characterization of the biomaterial and its different properties.

The same group continued with the addition of compounds into PHBV particles: They added the antibiotic ceftiofur using rats as a model and demonstrated the controlled release of ceftiofur into the bloodstream with levels over the minimum inhibitory concentration (MIC) for 17 days after injection, without adverse effects. The effect was longer than non-encapsulated antibiotics when challenged with *Salmonella typhimurium* and several parameters were measured, such as altered breath intensity, ruffled fur and persistent chills, among other properties. The combination of the slow release of ceftiofur from the particle, low toxicity, and high efficiency makes ceftiofur-PHBV a good candidate for applications in the veterinary industry [33]. High activity of PHBV depolymerase was shown in *S. omiyaensis SSM 5670*, showing a positive industrial potential to promote the degradation of PHBV products around room temperature [34], aiming to play a part in the genetic engineering of PHA-producers.

Searching to cheapen the cost of PHA particles, the waste ash from rice husk (RHA), i.e. the hard-protecting coverings of grains of rice, was used as a filler in polymer matrices to replace conventional fillers, such as talc. By twin-screw extrusion and injection molding, they were able to apply different percentages of the ash in the PHB composite formulation. PHB+ rice husk ash had better biodegradable properties than PHB/talc, while the other properties remained almost unchanged. The use of a by-product from the rice industry as a filler for biodegradable polymers should help to reduce environmental impact [35].

On PHBV-ceftiofur particles, in 2015, interesting features were described: Empty PHBV microparticles had more stability than the antibiotic-loaded ones in a buffer solution. Also, they described the ceftiofur release mechanism as based mainly on polymer matrix degradation. The Ceftiofur-PHBV nanoparticle was prepared as a simple emulsion, aiming to reduce the overall size of the particle and improving entrapment efficiency, stability and *in vitro* performance [36]. Also continuing with the use of the coffee wastes (10 to 20%) to enhance PHB based composites properties, the thermal stability of the composite was improved with the inclusion of the coffee residues, but its crystallinity decreased with the addition of the coffee parchment. Tensile and Izod strength improved with a 10% and 20% addition of parchment, concerning PHB. Also, a higher concentration of the coffee waste particles led to an increase in the water absorption of the composite [37].

Research with SPIONs continued, allowing for localization, direction and controlled concentration of drugs within the particle. PHBV nanoparticles functionalized with SPIONs carrying the antibiotic ceftiofur (PHBV/SPION/CEF) could be used in multiple biomedical applications, such as diagnosis and treatment of cancer and its associated bacterial infections. The nanoparticle was spherical-shaped with a core-shell structure with a high encapsulation efficiency, forming a nanocomposite and revealing antimicrobial properties against *Escherichia coli*, accompanied by low cytotoxicity, making PHBV/SPION/CEF nanoparticles suitable as a drug delivery system [38], while 2016 showed us the study about blends of microspheres of PHB/PCL for the controlled release of 8 the pesticide cypermethrin, in which PCL (polycaprolactone) influenced heavily the porosity of the microspheres. They successfully encapsulated the compound, and with a higher content of PCL, the crystallinity degree of polymers decreased, resulting in a faster release of the pesticide [39]. Photodegradation and photostabilization of two commercial grades of the 3PHB biopolymer were evaluated and both samples were destroyed by the exposure to UV radiation, damaging the surface color, reduction of molecular size and mechanical properties. Photostabilization was achieved by the injection of a UV absorber/antioxidant, resulting in a higher UV stability of the PHB [40].

2017 had three different research lines related to PHAs: Firstly, it showed us in detail the uptake mechanisms of PHBV nanoparticles in two epithelial cell lines: SKOV-3 (Ovarian cancer cells) and HeLa (Cervical cancer cells), by analyzing the size, surface charge, and shape of the PHBV particles, together with intracellular drug incorporation in both cell lines. The selected cell lines showed different uptake mechanisms: while HeLa cells internalization was based on an endocytic pathway, SKOV-3 didn't show any similar pattern, but the final fate of the PHBV nanoparticles was shared in lysosomes and degraded, showing how important is cell line selection to use nanoparticles for drug delivery [41]. Continuing with the search of cheap fillers for PHA composites, sisal natural/coconut fibers were evaluated, aiming to diminish environmental impacts produced by the fiber remnants. Composites with 10 to 15% of fibers were done by compression molding in a hydraulic press. Thermal stability and strength values remained unchanged between PHB/fibers and PHB. The research showed an alternative to reuse (and dispose of) agricultural residues, making the process cheaper, considering that the PHB required

would be less when blended with fibers. Finally, they suggest using the PHB+fiber blend as tubes for planting seedlings of vegetables and fruits in agriculture, as well as in packaging trays to substitute polystyrene [42]. Also, this year surprised us with the first report on the microbial coproduction of mcl-PHAs and CdS (Cadmium) Quantum Dots in *P. putida KT2440*, used as a cell factory in batch cultures. The synthesized PHA was present in the cell cytoplasm, whereas CdS-nanocrystals were located within the periplasmic space. In an interesting feature, and by using standard PHA-extraction procedures, the bio-synthesized QDots were not affected by the PHA extraction method and remained associated with the biomass. The resulting PHAs showed no traces of CdSQDots [43].

At the end of this revision, the surprising number of four research papers could be found for 2018: The effect of heat cycling on the preparation of PHB/TiO₂ blends, i.e. heating and cooling rates, showing that a higher cooling rate produces a lower melt crystallization temperature, while a higher heating rate turns into a higher cold crystallization temperature. The research group did other tests to the PHB/TiO₂ and their results suggest that the PHB microstructure can be controlled by filler content and adjusting the heating/cooling rate by its composition [44]. As a new filler, the synthesis of PHB calcium carbonate (CaCO₃) biocomposites from Rhea (*Rhea americana*) eggshells as a filler was described, by the insertion of calcined eggshell powder into a PHB solution for film preparation via solvent casting. The non-modified filler produced a greater porosity at the surface of the biocomposite films and with the calcined filler, homogeneous films with reduced porosity were obtained. From the analysis, they observed that the filler inserted into the PHB matrix can catalyze the biodegradation process in different ways [45].

Another novel filler was tested by a composite made of PHB/20% babassu compounds. The babassu palm (*Attalea speciosa*) grows in the Amazonas Region. They gave a detailed study by DSC of the non-isothermal melt crystallization of PHB in PHB/20% vegetable fiber (babassu) [46] and finally, the characterization of electrospun fibers obtained from bacterial PHBs. They used the bacterial strain LB400 with xylose and mannitol as the sole carbon sources. From both carbon sources, the surface morphology of the microfibers was different, indicating that carbon source may determine the fiber structure and properties, even if in both cases PHB is produced, and currently, they continue with the studies to analyze the physicochemical and mechanical properties of these PHB-based microfibers [47].

Conclusion

In general terms, South American research based on polyhydroxyalkanoates is wide, but the idiomatic barriers and the use of non-English databases, such as SciELO can undermine the possibilities of making their research known. Because of that, the authors of this review hope that the manuscript will be a starting point for fruitful collaborations between laboratories worldwide.

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Conflict of Interest

The authors declare no conflict of interest.

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