Highlights

- The review focuses on the challenges of scaling-up PHA production from wastes
- Combination of two or more waste streams could avoid additional synthetic streams
- Pre-treatments to adjust the waste to an appropriate feed stream is highly proposed
- Integration of PHA production in existing processes enhances their implementation
- PHA production from waste should be adapted to different seasonal waste streams

CHALLENGES OF SCALING-UP PHA PRODUCTION FROM WASTE STREAMS. A REVIEW

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Abstract

The search for new materials that replace fossil fuel-based plastics has been focused on biopolymers with similar physicochemical properties to fossil fuel-based plastics, such as Polyhydroxyalkanoates (PHA). The present paper reviews the challenges of scaling-up PHA production from waste streams during the period from 2014-2016, focusing on the feasibility of the alternatives and the most promising alternatives to its scaling-up. The reviewed research studies mainly focus on reducing costs or obtaining more valuable polymers. In the future, the integration of PHA production into processes such as wastewater treatment plants, hydrogen production or biodiesel factories could enhance its implementation at industrial scale.

Keywords:

Biopolymer; Operation mode; Pilot-scale; Polyhydroxyalkanoates; Waste biomass

1. Introduction

Plastic materials are increasingly used in many domestic and industrial fields. Their use is usually associated with the development of a comfortable life. The global production of plastic materials reached 299 million tons in 2013, where 98% of the total production corresponded to fossil fuel-based plastics obtained from petroleum (Mozejko-Ciesielska and Kiewisz, 2016). Moreover, global plastic production presents a growing trend, with an increase of 4% in the production of plastic materials in 2016 (Muhammadi et al., 2015). In fact, the growing trend in plastic production is expected to continue at least until 2020 (Anjum et al., 2016).

Fossil fuel-based plastics from petroleum are supported by a highly limited natural resource. The exploitation of petroleum for the current demand of plastic materials poses serious environmental concerns, such as global warming, human health risks or ecosystem toxicity (Harding et al., 2007). Furthermore, fossil fuel-based plastic materials are resistant to microbial degradation, and are accumulated in the environment with a highly polluting potential (Mozejko-Ciesielska and Kiewisz, 2016). In fact, 17 million tons of plastic waste were generated in the European Union in 2012 (Eurostat, 2017a). Plastic packing material accounted for around 88% of the plastic waste generated in the European Union in 2012, i.e., 15 million tons (Eurostat, 2017b). Therefore, the production and disposal of fossil fuel-based plastics are not sustainable and they can be considered as contrary to the European policies in relation to waste

management, emission reduction, and sustainable development (Directive 2008/98/EC on waste).

Even with the recycling and awareness policies, the situation shows a favorable scenario for the search of alternative technologies regardless of the petroleum that can satisfy the potential demand for plastic materials (Anjum et al., 2016). The search for new materials to replace fossil fuel-based plastics has focused on biopolymers with similar physicochemical properties to fossil fuel-based plastics. Biopolymers, or organic plastics, are plastic materials that can be produced from renewable sources or waste streams, and to reduce fuel consumption (Pittmann and Steinmetz, 2016). Moreover, biopolymers can reduce the environmental impact derived from plastic waste disposal due to the fact that the biodegradation time for biopolymers in the land surface under standard conditions is approximately two months (Hassan et al., 2013).

Indeed among the different biopolymers, polyhydroxyalkanoates (PHA) are polyesters produced by prokaryotic microbes as intracellular storage materials, which can be extracted and then formulated and processed for plastic production (Kushwah et al., 2016). Depending on the chain length, PHAs could be thermoplastic polyester, elastomers or even sticky resins composed of several R-hydroxyalkanoic acids that can be produced by numerous microorganisms (Koller and Brauneegg, 2015; Anjum et al., 2016). Nowadays, over 90 microbial species that generate PHA and about 150 different monomers of PHA have been identified (Zinn et al., 2001). PHAs have a great potential because of their interesting properties, high biodegradability, and their recyclable nature (Anjum et al., 2016; Kushwah et al., 2016). In fact, Essel and Carus (2012) showed that PHA production can save on average 2 kg CO₂ emitted and around 30 MJ of fossil resources for 1 kg PHA produced compared to fossil-fuel plastic production. Alternatively, accumulated PHA in the biomass could be used to enhance the

production of volatile fatty acids, hydrogen, and/or methane in anaerobic digestion processes (Wang et al., 2015a; Wang et al., 2015b).

Currently, commercial PHA production uses expensive raw materials and chemicals as sources of organic matter, which entails high costs at industrial scale. These costs must be offset to be an economically attractive alternative to fossil-fuel plastic production (Madkour et al., 2013). To enhance the profitability of the system and to facilitate its implementation in the plastic market, many operating alternatives have been proposed at lab-scale. One of the most promising alternatives is the use of industrial by-products and/or waste streams, i.e. agriculture feedstock, waste plant oils or wastewater, as sources of carbon for PHA production (Narodoslawsky et al., 2015). PHA production using waste streams can be considered an environmentally friendly management method (Anjum et al., 2016). However, pilot and industrial scale implementation require new technological advances to facilitate the employment of waste streams as raw material.

The present paper reviews the challenges of scaling-up PHA production that used waste streams as a carbon source in recent years, focusing on the feasibility of the alternatives and the most promising alternatives to its scaling-up. The review is focused on the 2014-2016 period, where a significant increase in scientific publications about PHA production can be found.

2. Scientific impact of PHA production

Figure 1 shows studies on PHA production from waste materials in the last 24 years. A huge increase in the studies has been produced over the 2014-2016 period. This increase is related to the need to introduce bioplastics in the market at competitive prices in comparison to fossil fuel-based plastics. Table 1 shows reviews and studies based on relevant steps of the PHA production process.

The timeline of these reviews shows the evolution in the development of PHA production research. During the 2012-2014 period, authors were mostly focused on strain improvement by selection, DNA extraction, and super productive strains (Mohammadi et al., 2012; Wang et al., 2014). In 2015, studies were mostly dedicated to reducing costs through the reuse of waste generated from PHA production or an increase in the generation of high added-value products (Wei et al., 2015). In addition, the identification of the weakest points in the process for implementation at industrial scale revealed the next research lines (Muhammadi et al., 2015; Chen et al., 2015a; Kaur and Roy, 2015; Liu et al., 2015). In 2016, authors had maintained attention on the improvement of genotypes and operational conditions of fermentation (Kawaguchi et al., 2016; Tai et al., 2016; Inoue et al., 2016). The integration of the PHA production into other industrial processes, use of mixed cultures and use of waste streams as a source of organic matter have been considered as main research lines (Anjum et al., 2016; Valentino et al., 2017; Koller et al., 2017; Jian et al., 2016; Mohan et al., 2016).

Table 1 shows that most authors have focused on reducing the cost of the PHA production process. Different strategies have been used to reduce costs such as genetic studies (Wang et al., 2014; Liu et al., 2015; Tai et al., 2016; Inoue et al., 2016); and optimization of the PHA purification/extraction step (Mohammadi et al., 2012; Liu et al., 2015; Kawaguchi et al., 2016); to obtain PHA compounds with higher economic value and/or promising new applications (Muhammadi et al., 2015; Anjum et al., 2016; Wang et al., 2014; Chen et al., 2015a; Kaur and Roy, 2015; Zia et al., 2016; Koller and Brauneegg, 2015); implementation of continuous processes (Anjum et al., 2016; Kaur and Roy, 2015; Valentino et al., 2017; Koller et al., 2017; Mohan et al., 2016, Koller and Muhr, 2014); to diversify the feed streams (Liu et al., 2015; Kawaguchi et al., 2016; Inoue et al., 2016; Valentino et al., 2017; Koller et al., 2017; Koller and Brauneegg,

2015; Mohan et al., 2016; Obruca et al., 2015); to re-use the bacterial biomass generated from PHA production (Wei et al., 2015); to reduce the energy consumption of the PHA production process (Mohammadi et al., 2012; Liu et al., 2015), integration into bio-refinery systems (Jiang et al., 2016), and to improve the yield of PHA production (Liu et al., 2015; Kawaguchi et al., 2016).

Previously described reviews and the increment in the scientific production research demonstrate the huge interest in PHA production from waste streams. However, a holistic and critical overview of the alternatives proposed in the literature is required in order to determine the most promising alternatives for a successful full-scale implementation. For this purpose, the present review identified and analyzed the most relevant parameters for PHA production from waste streams for each alternative proposed in the literature during the 2014-2016 period.

3. Parameters of PHA production

Figure 2 shows a simplified block diagram of the PHA production process by employing waste as a source of organic matter. This process consists of three main steps: pre-treatment, production and extraction/purification. Tables 2 and 3 summarize the main parameters reported in lab-scale and pilot-scale studies, respectively, published in the reviewed period. Processes which used less than 10 liters of work volume were considered lab-scale studies, whereas studies that used more than 10 liters of work volume were considered as pilot-scale.

3.1 Waste carbon source

Around 40-48% of the total production cost of PHA can be ascribed to raw materials (Schmidt et al., 2016). As raw material is the main carbon source for PHA synthesis and bacteria growing, using organic waste as a source of organic matter shows as a

promising alternative for the reduction in PHA production costs. Three major pathways have been described for PHA synthesis mechanism in literature. Two of them use sugars as a carbon source (Pathway I, Acetyl-CoA to 3-Hydroxybutyryl-CoA, and Pathway III, again fatty acid biosynthesis) and another third route uses fatty acids as a carbon source (Pathway II, fatty acid degradation) (Anjum et al., 2016). Therefore, sugar and/or fatty acid containing wastes could be best candidates to be used in PHA production. Authors such as Gómez-Cardoso et al. (2016) define the terms sugar waste (i.e. wastes rich in simple carbohydrates such as glucose, sucrose, fructose, saccharose or stachyose) and fatty waste (i.e. wastes with high contents in fatty acids such as myristic, palmitic, stearic, oleic, linoleic, linolenic butyric, acetic or propionic acids) for classifying the wastes in accordance with the carbon source composition. Yields around 0.6–0.8 g/g were reported for fatty substrates, which are higher than those for sugars 0.3–0.4 g/g (Cruz et al., 2016). In addition, other wastes with highly biodegradable organic matter can be good candidates due to their organic material and can be easily transformed into readily consumable carbon for PHA production through physicochemical or biologic pre-treatments, i.e. simple carbohydrates or volatile fatty acids (VFAs) such as acetate, propionate, butyrate or lactate.

Regarding of the period under study, different kinds of wastes have been reported as the carbon source for PHA production (Tables 2 and 3). Depending on their origin, the waste streams have been classified as agro-alimentary, industrial not agro-alimentary, food, urban wastes treatment plant, and synthetic substrate.

Agro-alimentary wastes include: vegetal solid wastes, such as rice bran (Oh et al., 2015), pea-shells (Patel et al., 2015; Kumar et al., 2016), chicory roots (Haas et al., 2015), potato peels, apple pomace, onion peels (Kumar et al., 2016), grape pomace (Follonier et al., 2015), animal farm waste, poultry litter (Bhati and Mallick, 2016), and

industrial waste water streams, such as wastewater from the three-phase olive oil extraction process (Alsafadi and Al-Mashaqbeh, 2017; Koumentza et al., 2015; Campanari et al., 2014; Ntaikou et al., 2014), leguminous processing waste water and fruit processing waste water (Elain et al., 2016), and candy bar factory waste water (Tamis et al., 2014). Additionally, low and medium added value by-products such as cheese whey (Gómez-Cardozo et al., 2016; Valentino et al., 2015a; Valentino et al., 2015b; Pais et al., 2016; Colombo et al., 2016) and olive oil distillate (Cruz et al., 2016), respectively, have been studied.

Food wastes comprise: solid wastes, such as spent coffee grounds (Cruz et al., 2014) and food waste composite (including boiled rice, cooked vegetables, un-cooked vegetables, cooking oil, vegetable peelings, cooked meat, boiled spices) (Amulya et al., 2015), and a liquid waste such as used cooking oil (Gómez-Cardozo et al., 2016; Cruz et al., 2015; Cruz et al., 2016).

All industrial, not agro-alimentary wastes are generated by biodiesel manufacturing: crude glycerol (Gómez-Cardozo et al., 2016; Bera et al., 2015; Ribeiro et al., 2016; Ray et al., 2016; de Paula et al., 2016); oil cake hydrolysate (Bera et al., 2015) and biodiesel fatty acid by-product from glycerol purification (Cruz et al., 2016). In addition, carboxylic acids contained in glycerol anaerobic digestion effluent from 1,3-propanediol production were successfully studied in Pan et al., (2016) as carbon source. Anaerobic digestion effluent consists of 1,3-propanediol and carboxylic acids, involving a difficult separation stage (Pan et al., 2016).

Urban waste treatment plant wastes include carbon sources from both municipal solid and wastewater treatment plants: activated wastewater sludge (Cha et al., 2016; Morgan-Sagastume et al., 2015; Jia et al., 2014), the organic fraction of municipal solid

waste leachate (Korkakaki et al., 2016), activated wastewater sludge combined with the organic fraction of municipal solid waste (Basset et al., 2016).

Synthetic substrates have been used by several authors to determine the PHA production potential of different wastes. The influence of high salinity in industrial wastewater was studied by Rodriguez-Contreras et al. (2016) and Palmerio-Sanchez et al. (2016). Rodriguez-Contreras et al., (2016) used a glucose solution with different concentrations of NaCl to evaluate the effect of salt concentration in PHA production. In Palmerio-Sanchez et al. (2016), the influence of transient concentration of sodium NaCl was studied using a VFAs mixture and simulating the seasonal NaCl concentration in the wastewater of a fish-canning industry. The influence of the pH in the feed substrate was studied for synthetically activated wastewater sludge by Amulya et al. (2016). In Reddy et al. (2015), PHA production is proposed as a method for the mitigation of toxic compounds in industrial wastewaters (leather processing, textiles, pharmaceutical, and oil plants), in addition to obtaining high added-value compounds. A mineral synthetic medium including alkylphenols or mono-aromatic hydrocarbons was used as synthetic industrial wastewaters (Venkateswar-Reddy et al., 2015). The production of PHA was evaluated by Schmidt et al. (2016) using a glucose solution in order to simulate the carbon concentration usually found in agro-industrial wastes. VFAs solutions were employed by several authors as simulated wastes after acidifying fermentation (Chen et al., 2015b; Chen et al., 2016a; Venkateswar-Reddy et al., 2016; Fradinho et al., 2016). Finally, to evaluate the capacity of PHA production using raw gas effluents containing CO₂ as carbon source, pure CO₂ (Kaewbai-ngam et al., 2016) and CO₂-enriched air (Bhati and Mallick, 2016) were used as substrate and co-substrate with poultry litter, respectively.

Figures 3 and 4 show the percentage of references and the temporal evolution of the number of references both for the period under study (2014-2016 period) and per kind of waste, respectively.

Agro-alimentary wastes represent most of the employed substrates in the reviewed period, at 36%. It can be explained by the high availability and the wide diversity of wastes than can be included in this category, from vegetables solid wastes to industrial wastewater. Cheese whey, with five references, is the most referenced waste. Only the supply of the wastewater from the three-phase olive oil extraction process could be compromised by the implementation of two-phase olive extraction process around the world. In fact, in Spain, the main olive oil producer in the world, the production of olive oil by the three-phase process is negligible (Rincón et al., 2012). At pilot plant scale, results for liquid wastes, industrial wastewater (candy bar production) and olive oil wastewater, were published for agro-industrial wastes (Ntaikou et al., 2014; Tarnis et al., 2014). In addition, the simultaneous production of hydrogen and PHA is proposed as a promising solution for managing some solid wastes (Patel et al., 2015; Kumar et al., 2016).

Wastes generated by biodiesel manufacturing, which comprise all industrial, not agro-alimentary wastes, are the second most studied kind of waste with 7 references (18%) due to their high concentration in VFAs. The integration of PHA production into the biofuel industry could reduce production costs in both processes, and facilitate the scaling-up of the PHA production process (Jiang et al., 2016). Only lab-scale works were reported with this kind of wastes.

Food wastes represent 11% of reviewed studies. Used cooking oil is the most studied food waste at lab-scale due to their composition in medium and long chain fatty acid units that can act as precursors for different types of PHA (Cruz et al., 2016). There are

two references at pilot-scale for food waste, i.e. grape pomace (Follonier et al., 2015), and a mixture of solid food waste with simultaneous hydrogen production (Amulya et al., 2015). The other solid waste under study at lab-scale, spent coffee grounds (Cruz et al., 2014), could be not easily collected for a full-scale plant, compromising the success of a process based on this waste carbon source. The success of a process based on food wastes would be linked to an effective and economically viable method of collection.

Urban waste treatment plant wastes also represent 11% of reviewed studies. The integration of the PHA production into urban waste treatment plants could keep a constant and great carbon source without extra cost for waste collection, which are some of the main challenges for scaling-up the PHA production processes (Pittmann and Steinmetz, 2016). That could compensate for the complex composition of the wastes with respect to other alternatives. Two pilot-scale studies were published in the period under study employing wastewater (Morgan-Sagastume et al., 2015) and activated wastewater together with the organic fraction from solid wastes (Jia et al., 2014).

The other reviewed studies, around 24%, reported the use of synthetic wastes. As described above, the potential of different wastes streams for PHA production was evaluate by studying the effect of pH, high salinity, different concentrations of VFAs and presence of organic toxic compounds at lab-scale. In the case of organic toxic compounds, PHA production could reduce the treatment costs of wastewaters with these compounds, which can be treated mainly by physicochemical processes with a high cost and inappropriate volumes for large wastewaters (Venkateswar-Reddy et al., 2015). Additionally, CO₂-rich gas streams were studied as carbon source as a promising global warming mitigation system, i.e. by integration of PHA production into fossil fuel based power plants.

The studies for the different kinds of wastes show an increment from 2014 to 2016. The proposal of new kinds of wastes, such as CO₂-rich streams or high salinity waste waters, and the promotion of useful new knowledge will be necessary for a successful development of a competitive commercial process.

3.2. Substrate pre-treatment

The physicochemical characteristics of the waste materials can make it necessary to include a previous treatment to adapt them to the requirements of the PHA production process. This treatment is previous to PHA production, so it is called the pre-treatment phase. This phase might be used to increase the carbon sources available (Pittmann and Steinmetz, 2016; Haas et al., 2015; Oh et al., 2015; Kourmentza et al., 2015; Campanari et al., 2014; Ntaikou et al., 2014; Tamis et al., 2014; Valentino et al., 2015b; Pais et al., 2016; Colombo et al., 2016; Amulya et al., 2015; Bera et al., 2015; Pan et al., 2016; Morgan-Sagastume et al., 2015; Jia et al., 2014; Korkakaki et al., 2016; Basset et al., 2016; Chen et al., 2015b; Chen et al., 2016b), dilute the concentration of organic matter (Pittmann and Steinmetz, 2016; Bhati and Mallick, 2016; Alsafadi and Al-Mashaqbeh, 2017; Ntaikou et al., 2014; Amulya et al., 2015; Ray et al., 2016; Jia et al., 2014), regulate the pH (Patel et al., 2015; Kourmentza et al., 2015; Amulya et al., 2015), control the temperature (Kourmentza et al., 2015; Campanari et al., 2014), sterilize waste material (Oh et al., 2015; Kourmentza et al., 2015; Colombo et al., 2016), and/or remove suspended solids (Oh et al., 2015; Haas et al., 2015; Patel et al., 2015; Kumar et al., 2016; Kourmentza et al., 2015; Campanari et al., 2014; Ntaikou et al., 2014; Valentino et al., 2015a; Amulya et al., 2015; Morgan-Sagastume et al., 2015; Jia et al., 2014; Basset et al., 2016; Follonier et al., 2015). In addition, some waste materials were solids such as coffee grounds (Cruz et al., 2014), chicory roots (Haas et al., 2015), poultry waste (Bhati and Mallick, 2016) or boiled rice, cooked vegetables, uncooked

vegetables (spoiled), vegetable peel, cooked meat and boiled spices (Amulya et al., 2015). In these cases, the extraction of an organic liquid phase was necessary. The organic liquid phase could be achieved dissolving the organic fraction in a water medium (Bhati and Mallick, 2016; Hass et al., 2015), using a supercritical CO₂ extraction (Cruz et al., 2014) or masticating by an electrical blender (Amulya et al., 2015).

In order to increment the available carbon source, authors have used mostly two technics: chemical hydrolysis (Oh et al., 2015; Kumar et al., 2016; Pais et al., 2016; Bera et al., 2015; Chen et al., 2016a) and anaerobic fermentation (Pittmann and Steinmetz, 2016; Kourmentza et al., 2015; Campanari et al., 2014; Ntaikou et al., 2014; Tamis et al., 2014; Valentino et al., 2015b; Colombo et al., 2016; Amulya et al., 2015; Pan et al., 2016; Morgan-Sagastume et al., 2015; Jia et al., 2014; Korkakaki et al., 2016; Basset et al., 2016; Chen et al., 2015b). The waste materials which require the available carbon source increment are mostly from the food industry i.e., rice bran (Oh et al., 2015), pea-shells and onion (Kumar et al., 2016), cheese whey (Valentino et al., 2015b; Pais et al., 2016; Colombo et al., 2016), sugars (Chen et al., 2015b; Chen et al., 2016a), boiled rice, cooked vegetables, spoiled un-cooked vegetables, cooking oil, vegetable peels, cooked meat, boiled spices (Amulya et al., 2015), and waste treatment i.e., urban wastewater (Pittmann and Steinmetz, 2016; Morgan-Sagastume et al., 2015; Jia et al., 2014; Basset et al., 2016), olive mill wastewater (Kourmentza et al., 2015; Campanari et al., 2014; Ntaikou et al., 2014), leachate from urban solid waste (Korkakaki et al., 2016). On the one hand, a clear relation between the waste material and the selected hydrolysis technique was not observed. On the other hand, the anaerobic fermentation was the most widely used technique when the waste material was from a waste treatment plant. This fact could be explained because the PHA process was integrated

into the waste treatment process, using installations already built, i.e. the anaerobic fermenter. This seems a key advantage to reducing both PHA production costs and the costs of disposal waste.

The dilution operation was mainly used for waste materials from industrial processes, such as crude glycerol (Ray et al., 2016) and the effluent from the hydrogen production process (Patel et al., 2015), or waste treatment processes such as olive mill wastewater (Alsafadi and Al-Mashaqbeh, 2017; Kourmentza et al., 2015; Ntaikou et al., 2014) and sewage sludge (Pittmann and Steinmetz, 2016; Jia et al., 2014). Dilution was also applied when the waste concentration could produce a growth inhibition on the biomass used in the PHA production process.

In the lab-scale studies, 53% used some substrate pre-treatment operation (Table 2). In these studies, real field waste materials were used as carbon source. Alternatively, 47% of the lab-scale studies did not use any substrate pre-treatment operation (Table 2). In these studies, the carbon source used was always solid-free. Two main reasons to not require any pre-treatment have been observed. On one hand, the authors used a synthetic waste as a carbon source (Schmidt et al., 2016; Cha et al., 2016; Palmeiro-Sánchez et al., 2016; Amulya et al., 2016; Chen et al., 2016a; Venkateswar-Reddy et al., 2016; Fradinho et al., 2016) or, on the other hand, the waste used was rich in sugars or VFA (Cruz et al., 2015; Cruz et al., 2016; Elain et al., 2016; de Paula et al., 2016). All pilot-scale studies showed a substrate pre-treatment (Table 3). This is due to the fact that in the pilot-scale studies the authors used real waste streams as a carbon source. The necessity of a pre-treatment step is an important challenge for the scaling-up of PHA production due to the additional costs. Therefore, the optimization of the pre-treatment processes is necessary to avoid unnecessary costs. Alternatively, the addition of extra nutrient source could replace the pre-treatment step in some cases.

The number of operations needed in the substrate pre-treatment phase is related to the waste selected as the carbon source. Therefore, a waste which is rich in available carbon source or one which can be easily enriched by a pre-treatment phase seems better than a solid waste, which needs other operations that could complicate the flow sheet of the process. The industrial production of PHA (Anjum et al., 2016; Kushwah et al., 2016) used sugar and glucose extracted from plant sources is an attempt to reduce costs. The pre-treatment phase seems a set point for the scaling-up of the process. **3.3. Culture**

The culture used for PHA production strongly influences the operation procedure, as well as the investment and operation cost (Kushwah et al., 2016). The different cultures were classified as pure or mixed. Pure microbial cultures are the most widely employed at lab-scale (Table 2), although mixed cultures have been receiving growing attention in the last years. Concretely, 61% of the revised manuscript in the period 2014-2016 used pure culture at lab-scale (Table 2). Prior to the cultivation and PHA generation, pure strain cultures were usually maintained by the addition of different mixtures of synthetic compounds such as agar, tryptone, yeast extract or other nutrients, in accordance with the strain requirements (Cruz et al., 2015; Ribeiro et al., 2016; Pan et al., 2016). The genus *Bacillus* was the most employed in pure culture (Patel et al., 2015; Ribeiro et al., 2016; Pan et al., 2016; Rodriguez-Contreras et al., 2016; Venkateswar-Reddy et al., 2015). Some bacteria of the genus *Cupriavidus*, mainly *C. necator*, were also extensively employed for PHA production (Schmidt et al., 2016; Cruz et al., 2014; Cruz et al., 2015; Ribeiro et al., 2016; Haas et al., 2015). Genera *Haloferax* and *Halomonas* were also employed at lab-scale, but to a lesser extent than genus *Bacillus* and genus *Cupriavidus* (Alsafadi and Al-Mashaqbeh, 2017; Elain et al., 2016; Pais et al., 2016; Bera et al., 2015). Indeed among these genera, the most frequently employed prokaryotic microbes at lab-scale were *Haloferax mediterranei* (Alsafadi and Al-

Mashaqbeh, 2017; Pais et al., 2016). These genera required a high salt concentration to grow (Elain et al., 2016). Therefore, high salinity conditions could limit the growth of other microorganisms by natural selection, which could be more interesting than the maintenance of aseptic conditions. Other genera can be selected as pure strains, although their uses were minor, e.g. *Ralstonia*, *Escherichia* (Oh et al., 2015), *Pseudomonas* (Cruz et al., 2015), *Hydrogenophaga* (Venkateswar-Reddy et al., 2016), *Nostoc* (Bhati and Mallick, 2016); or *Pannonibacter* (Ray et al., 2016). Moreover, *Escherichia coli* was the most commonly used microorganism for the genetic modification studies (Oh et al., 2015; Phithakrotchanakoon et al., 2013). At pilot-scale, the employment of pure culture was more restrictive, i.e. only 43% of the reviewed research studies described the use of pure cultures (Table 3). Genera *Pseudomonas* (Ntaikou et al., 2014; Follonier et al., 2015) and *Plasticumulans* (Tamis et al., 2014) were the genus employed at pilot-scale.

Mixed cultures for PHA production have been proposed due to the fact that they are simpler and less costly than the process with pure cultures, since sterile conditions and infrastructure for an axenic bioprocess are not required (Valentino et al., 2017). Indeed among the different mixed cultures reported in the Literature for the 2014-2016 period, the employment of wastewater activated sludge (WAS) was the most widespread (Tables 2 and 3) (Pittmann and Steinmetz, 2016; Cha et al., 2016; Korkakaki et al., 2016; Palmeiro-Sánchez et al., 2016; Amulya et al., 2016; Chen et al., 2015b; Chen et al., 2016a). WAS obtained from wastewater treatment plants could not be employed directly as culture for PHA production. It is necessary to fix nutrient and operation conditions for the selection of PHA accumulating cultures and their enrichment in the biomass (Korkakaki et al., 2016). The employment of WAS presents the additional advantage of integrating PHA production into the wastewater treatment processes

(Pittmann and Steinmetz, 2016). This integration allows ensuring the continuous availability of mixed cultures, as well as organic matter from wastewater treatment plant effluent, which could be employed as substrates for PHA production. The relevance of WAS as a culture is evidenced by its major employment at pilot-scale, i.e. 66% of the employed cultures were WAS (Amulya et al., 2015; Morgan-Sagastume et al., 2015; Jia et al., 2014; Chen et al., 2016a).

The necessity of aseptic conditions is an important disadvantage to the employment of pure cultures at full-scale. Therefore, mixed cultures could be more interesting for the scaling-up of PHA production processes due to the reduction in the cost compared to the maintenance of aseptic conditions. The integration of PHA production processes into wastewater treatment plants and the employment of WAS seems to be the most interesting alternative for scaling-up processes. For pure cultures, the limitation of undesirable microorganisms by natural selection could be more interesting than the maintenance of aseptic conditions.

3.4. Additional raw materials

Carbon source and total carbon load, nutrient concentration, C/N and C/P ratios, pH, and reactor environment are relevant parameters which influence the PHA production and the obtained compounds. In this sense, additional streams or chemicals were used in order to control them.

PHA yield and composition were found to be directly related to the composition of the substrate used (Venkateswar-Reddy et al., 2016). Therefore, several authors employed specific co-substrates with the aim to improve the bioconversion process to PHA and/or the final PHA composition.

The results of Patel et al. (2015) and Kumar et al. (2016) proved an effective enhancement of PHB (poly (3-hydroxybutyrate) yield by the addition of glucose as carbon source to fermented food wastes. The addition of tween-80 as co-substrate and surfactant showed a higher increment in toxic compounds (alkylphenols and mono-aromatic hydrocarbons) during the PHB production process (Venkateswar-Reddy et al., 2015).

As PHB application range has been limited due to its high crystallinity and low extension to break, several authors have proposed the addition of specific co-substrates in order to promote the incorporation of other units such as 3-hydroxyvalerate and 3-hydroxyhexanoate to the PHA composition. The final target is to obtain some copolymers with suitable properties for thermoplastic processing (Schmidt et al., 2016; Bhati and Mallick, 2016; Bera et al., 2015; de Paula et al., 2016). In Schmidt et al. (2016)- Propionic acid was added as co-substrate to induce the production of 3-hydroxyvalerate units. A higher ratio of propionic acid over glucose enhanced the 3-hydroxyvalerate fraction for PHB-co-3-hydroxyvalerate production (Schmidt et al., 2016). In Bera et al.(2015), the addition of seaweed derived crude levulinic acid (short-chain fatty acid), with crude glycerol and oil cake hydrolysate as waste carbon source, increased the cell PHA accumulation and altered the polymer composition, resulting in high levels of 3-hydroxyvalerate copolymer. This effect was not observed using pure seaweed derived levulinic acid. Additional constituents of crude acid, including formic acid, residual sugars, and dissolved minerals, played an important role in cell division and accumulation of PHA (Bera et al., 2015). Solutions of acetate, valerate and a glucose mixture were used as co-substrates with poultry litter and CO₂ as waste carbon sources in Bhati and Mallick (2016) for PHB-co-3-hydroxyvalerate production. Propionic acid and hexanoic acid were added to crude glycerol in de Paula et al. (2016)

to produce 3-hydroxyvalerate and 3-hydroxyhexanoate, respectively. At pilot-scale, Follonier et al. (2015) reported the addition of an equimolar solution of octanoic acid and 10-undecenoic acid as medium-chain-length PHA precursors.

The use of an additional carbon source has also been proposed with the aim to avoid the PHA production inhibition shown by some complex VFA-rich waste streams. These wastes have negative effects on biological activity due to, for example, the high concentration of inhibitor compounds such as ammonium (Korkakaki et al., 2016) or the presence of recalcitrant or complex carbon compounds (Basset et al., 2016). A synthetic VFA-rich solution was used as the only carbon source in the first stage of culture growing or enrichment. The waste stream was only used in the accumulation stage. Two lab-scale experiments employing activated wastewater sludge (Cha et al., 2016) and municipal solid waste leachate (Korkakaki et al., 2016), and one pilot-scale work employing olive mill wastewater (Ntaikou et al., 2014) included this additional stream were carried out.

Waste substrates available for PHA production contain varying levels of nutrients (N and P). A minimum concentration of nutrients is necessary for PHA production and accumulation but too high a concentration causes inhibition (Colombo et al., 2016). Moreover, optimal nutrient concentrations usually vary during the PHA process as it is carried out in different stages. In addition, the ratios C/N and P/N can condition the final composition of the obtained polymer (Valentino et al., 2015a). For example, phosphorus depletion led to lower PHB production, with 3-hydroxyvalerate remaining at the same level with respect to nitrogen depletion in Schmidt et al., (2016). In this sense, only some waste streams which have an appropriate C/N/P ratio can be used without the addition of nutrients (N and or P). This is the case of cheese whey (Colombo et al.,

2016) and some activated wastewater sludge (Morgan-Sagastume et al., 2015; Jia et al., 2014).

The full composition of the media plays an important role in PHA production/accumulation. The concentration of trace metals, i.e. Fe, B, Cu, Mn, Mo, Zn, and salinity can highly affect the PHA production yield (Alsafadi and Al-Mashaqbeh, 2017; Palmeiro-Sánchez et al., 2016; Chen et al., 2015b).

A specific inhibitor, allylthiourea, was added to the medium with the aim of limiting the possible influence of nitrification at laboratory (Colombo et al., 2016) and pilot-scales (Tamis et al., 2014). In Colombo et al. (2016), the substrate used was fermented cheese whey, which contained in itself the necessary N-nutrients, and the inhibitor was added to the media from the beginning of the experiment. In Tamis et al. (2014), the N-nutrients were supplied by adding a nutrient-enriched solution, and allylthiourea was added when NO_3^- or NO_2^- concentrations exceeded a set limit. In addition, in order to avoid foam generation, antifoam addition was reported in some small lab-scale experiments (Oh et al., 2015; Elain et al., 2016; Pais et al., 2016; Follonier et al., 2015; Haas et al., 2015).

As an alternative to the use of synthetic and expensive additional carbon source streams, other authors have proposed the mixture of waste carbon sources with different compositions to optimize the final substrate at lab scale. In Kumar et al. (2016) different mixtures of vegetables were studied with the aim to improve the PHA production by optimizing the VFA composition in the fermented effluent. In Basset et al. (2016) the PHA production yield was improved by using an additional waste carbon source such as the organic fraction of municipal solid waste (OFMSW). The OFMSW had a better C/N/P ratio than wastewater and more easily biodegradable biomass. Also, in Bathi and

Mallick (2016), the use of poultry litter containing N and P as co-substrate reduced the additional nutrient requirements for CO₂ metabolization.

The use of additional streams and chemicals has been widely used in the reviewed studies and has been shown to increase the cost of PHA production from wastes. Simpler synthetic carbon sources were added to improve the PHA yield and/or avoid culture growing inhibition. Specific VFAs were added to successfully obtain higher added value polymers at lab-scale with synthetic and real wastes. Nutrients were added to adjust the C/N/P ratio in order to improve the PHA yield and/or to optimize the polymer composition. Synthetic media or chemical additions were employed to maintain an optimal medium composition (trace metal concentration, salinity and/or pH).

The use of urban waste treatment plant waste production has a great advantage over the rest of wastes employed in terms of cost reduction because the possibility of saving extra cost due to additional raw material requirements. Urban waste treatment plant wastes can contain the necessary nutrients with acceptable C/N/P ratio and supply the medium for cell growth as has been proven at pilot-scale. Therefore, urban waste treatment plant waste production could be considered as a promising nutrient source to facilitate the scaling-up of the PHA production process. Also, the use of cheese whey could have some advantages over other wastes because it reduces the nutrient supply requirements due to its nutrient contents.

3.5. Operation mode

The PHA production process can be classified in three phases i.e., pre-treatment (phase I), culture growth / acclimation or culture enrichment (phase II) and PHA production / accumulation (phase III). Phase I commonly depends on the waste carbon source used

as mentioned above. Therefore, only PHA production processes which used a waste carbon source which required a pre-treatment showed phase I (Pittmann and Steinmetz, 2016; Oh et al., 2015; Patel et al., 2015; Kumar et al., 2016; Bhati and Mallick, 2016; Alsafadi and Al-Mashaqbeh, 2017; Kourmentza et al., 2015; Campanari et al., 2014; Valentino et al., 2015a; Valentino et al., 2015b; Pais et al., 2016; Colombo et al., 2016; Cruz et al., 2014; Bera et al., 2015; Ray et al., 2016; Pan et al., 2016; Korkakaki et al., 2016; Basset et al., 2016; Chen et al., 2015b; Haas et al., 2015).

Phase II has the goal of achieving enough active biomass capable of producing and accumulating the maximum PHA. For this goal, authors have used two different strategies according to the type of culture used. PHA production processes which used pure cultures (Schmidt et al., 2016; Gómez-Cardozo et al., 2016; Oh et al., 2015; Patel et al., 2015; Kumar et al., 2016; Bhati and Mallick, 2016; Alsafadi and Al-Mashaqbeh, 2017; Elain et al., 2016; Pais et al., 2016; Cruz et al., 2015; Bera et al., 2015; Ribeiro et al., 2016; Ray et al., 2016; de Paula et al., 2016; Pan et al., 2016; Rodriguez-Contreras et al., 2016; Venkateswar-Reddy et al., 2015; Haas et al., 2015; Venkateswar-Reddy et al., 2016) usually grew and acclimated the strains used before phase III. However, PHA production processes which used mixed cultures (Kourmentza et al., 2015; Campanari et al., 2014; Valentino et al., 2015a; Valentino et al., 2015b; Colombo et al., 2016; Cha et al., 2016; Korkakaki et al., 2016; Basset et al., 2016; Palmeiro-Sánchez et al., 2016; Amulya et al., 2016; Chen et al., 2015b; Chen et al., 2016a) enriched the culture before phase III. The main difference between these strategies was that pure culture processes were done with PHA producer strains according to the bibliography. So these processes needed phase II only to achieve the sufficient amount of active biomass. While the mixed culture processes required the selection and enrichment of the strains capable of producing PHA from the mixed seeds used.

In order to grow the pure culture, most authors have used a synthetic medium with an easily degradable carbon source (Oh et al., 2015; Patel et al., 2015; Alsafadi and Al-Mashaqbeh, 2017; Ribeiro et al., 2016; Rodriguez-Contreras et al., 2016; Venkateswar-Reddy et al., 2015). Additionally, some pure cultures have been acclimated previously to phase III by the nutrient limitation medium i.e., C/N or C/N/P ratio regulation (Schmidt et al., 2016; Patel et al., 2015; Alsafadi and Al-Mashaqbeh, 2017; Ray et al., 2016). Phase II mostly showed a C/N or C/N/P ratio regulation (Patel et al., 2015; Bhati and Mallick, 2016; Kourmentza et al., 2015; Campanari et al., 2014; Elain et al., 2016; Tamis et al., 2014; Valentino et al., 2015b; Colombo et al., 2016; Amulya et al., 2015; Ribeiro et al., 2016; Pan et al., 2016; Cha et al., 2016; Basset et al., 2016) and because of this, it had been reported that nitrogen and phosphorus restriction in the culture could improve PHA accumulation.

The culture enrichment, carried out in phase II, has mostly been carried out by the feast/famine regime in the PHA production processes with mixed cultures (Kourmentza et al., 2015; Campanari et al., 2014; Valentino et al., 2015a; Colombo et al., 2016; Korkakaki et al., 2016; Palmeiro-Sánchez et al., 2016; Amulya et al., 2016; Chen et al., 2015b; Chen et al., 2016a; Fradinho et al., 2016). In this regime, the feeding was added intermittently. The carbon source was added in the feast phase in which microorganisms grow, followed by the phase without carbon source addition i.e., the famine phase. Thus, a selection pressure was generating to select microorganisms capable of producing and accumulating PHA (Fradinho et al., 2016). Alternatively, the acclimation of the biomass has been regulated by the control of the feeding mode which produced the selection pressure mentioned above (Cha et al., 2016).

PHA production and accumulation occurred in Phase III. The carbon source used in the culture was mainly stored as PHA in microorganisms. Phase III was usually separated

from Phase II in a different reactor. In fact, 75% of the lab studies reported these phases as separated (Table 2). Authors which showed Phase II and Phase III in the same reactor have used a pure culture, except Fradinho et al. (2016), who used a photosynthetic consortium of bacteria and algae. Studies which joined Phase II and Phase III used algae or photosynthetic bacteria (Bhati and Mallick, 2016; Fradinho et al., 2016; Kaewbai-ngam et al., 2016) or bacteria cultures with high production yield of PHA/cell growth (Gómez-Cardozo et al., 2016; Pais et al., 2016; Cruz et al., 2014).

Previous studies reported that PHA structure is directly related to the composition of the substrate used (Venkateswar-Reddy et al., 2016). The metabolic pathway involved in the PHA synthesis determines the polymer produced. Pathways involved in fatty acid, glucose, sucrose and fructose metabolism generate different hydroxyalkanoate monomers utilized in PHA biosynthesis (Anjum et al., 2016). Polymer production with different properties, suitable for different applications has been studied (Cruz et al., 2016). A proportional relationship was observed between the composition of fatty acids and the molecular structure of PHAs: a greater percentage of fatty acids of medium and long chains leads to a greater molar percentage of building blocks with medium and long chains (Ribeiro et al., 2016). Therefore, the waste carbon source, pre-treatment, culture or even the additional raw materials might be selected to improve the value of the polymers produced (Schmidt et al., 2016; Cruz et al., 2016; Elain et al., 2016; Valentino et al., 2015a; Pais et al., 2016; Ribeiro et al., 2016; de Paula et al., 2016; Pan et al., 2016; Korkakaki et al., 2016; Basset et al., 2016; Venkateswar-Reddy et al., 2016).

Lab-scale studies showed two different operation modes i.e., batch and continuous operations. Only 8% was carried out at continuous operation mode (Table 2). This fact could be explained because most lab studies were focused on preliminary studies to

obtain results and information about the process and not the scalability. In the pilot-scale studies, the PHA production processes studied were carried out in the 3 phases (Table 3) described before. Pilot-scale studies showed mostly mixed cultures except Tamis et al. (2014), who used a pure culture. Most pilot-scale studies showed Phase II as biomass enrichment (Table 3), even the pure culture process used by Tamis et al. (2014). Reviewed studies observed at pilot-scale (Table 3) were carried out in continuous mode operation. The continuous operation mode is the desirable one since it requires little monitoring. Inadequate monitoring in a continuous PHA production process could result in microbial contamination, disruption of microbial cells by agitation and aeration, undesirable cell growth on the walls or other surfaces of the bioreactor (Koller and Muhr, 2014). A correct adjustment of the process design to the kinetic particularities of selected microbes on applied substrates is necessary to remedy these challenges, as well as to achieve high productivity and complete substrate conversion in continuous mode PHA processes (Koller and Muhr, 2014). Moreover, the correct control of the operational parameters could ensure the obtaining of biopolymers with exactly tailored composition on the monomeric level for the fine-tuning of material characteristics (Koller and Muhr, 2014). Although the current state of the technology implies that most of studies were operated in batch sequences (Table 2), continuous operation periods of more than one year have been reported (Chen et al., 2015a; Morgan-Sagastume et al., 2015). These studies showed that PHA production can be supplied with normal high flow streams and operate continuously. According to the reviewed studies (Table 2 and Table 3), the increment of the pilot-scale studies in this technology seems necessary since only 12% (Table 3) could show significant results to the scalability at industrial scale.

The operation mode was influenced by the waste carbon source, pre-treatment phase, culture, and additional raw materials. The studies reviewed were focused on the reduction of the PHA production cost and yield increase. In order to reduce the production costs, which is the main challenge of the PHA production, and in addition to the waste utilization as a carbon source, the integration of the PHA process into an industrial process was studied (Gómez-Cardozo et al., 2016; Cruz et al., 2016; Patel et al., 2015; Kumar et al., 2016; Alsafadi and Al-Mashaqbeh, 2017; Kourmentza et al., 2015; Campanari et al., 2014; Ntaikou et al., 2014; Tamis et al., 2014; Amulya et al., 2015; de Paula et al., 2016; Cha et al., 2016; Morgan-Sagastume et al., 2015; Korkakakiet al., 2016; Basset et al., 2016, Jiang et al., 2016). Biogas production, hydrogen production, biodiesel manufacturing, and wastewater treatment were the main industrial processes which showed a favorable implementation. The union of phases II and III has been another viable option to reduce costs (Gómez-Cardozo et al., 2016; Cruz et al., 2016; Bhati and Mallick, 2016; Cruz et al., 2014; Fradinho et al., 2016; Kaewbai-ngam et al., 2016), but there are not studies at pilot-scale. For the increase in PHA production, two strategies have been studied. One of them was focused on the optimization of the C/N/P ratio of the feed streams to achieve an active biomass capable of producing the maximum amount of PHA. The other strategy was focused on the value increase of the polymers or copolymers produced in the process (Kushwah et al., 2016; Madkour et al., 2013; Cruz et al., 2016). In order to increase the value of the produced polymers, the composition of the feed stream and/or additional raw material have been studied.

3.6. Yield and PHA accumulation

A great variety of expressions to report the production of PHA have been used in the literature. Moreover, there is no clear consensus on the units of these expressions. To

facilitate the comparison of the results, as well as the scaling-up, research studies should include some parameters such as PHA accumulation percentage in the biomass, PHA yield in relation to the added substrate and PHA yield in relation to the added waste. The report of PHA yield in relation to the added waste was not extended and was only reported in around 25% of the reviewed research (Pittmann and Steinmetz, 2016; Cruz et al., 2016; Oh et al., 2015; Alsafadi and Al-Mashaqbeh, 2017; Campanari et al., 2014; Pais et al., 2016; Cruz et al., 2015; Pan et al., 2016; Venkateswar-Reddy et al., 2015). PHA yield in relation to the added waste could be interesting to evaluate, individually, with respect to the production from waste in processes with additional carbon sources.

Accumulation capacity values varied in a wide range, i.e. from 7.5% to 97.2% (Table 2) (Oh et al., 2015; Kumar et al., 2016). Most of the reviewed research reported accumulation capacity values between 60% and 80% (Schmidt et al., 2016; Patel et al., 2015; Bhati and Mallick, 2016; Kourmentza et al., 2015; Haas et al., 2015; Elain et al., 2016; Valentino et al., 2015a; Cruz et al., 2014; Cruz et al., 2015; Bera et al., 2015; Ribeiro et al., 2016; Ray et al., 2016; Cha et al., 2016; Korkakaki et al., 2016; Chen et al., 2015b; Venkateswar-Reddy et al., 2016). The high accumulation capacity values were clearly related to the employment of synthetic co-substrates (Kourmentza et al., 2015; Ribeiro et al., 2016; Cha et al., 2016; Venkateswar-Reddy et al., 2016), additional streams to regulate the process (Oh et al., 2015; Patel et al., 2015; Elain et al., 2016; Korkakaki et al., 2016), additional streams as extra nutrient source (Schmidt et al., 2016; Patel et al., 2015; Bhati and Mallick, 2016; Valentino et al., 2015a; Bera et al., 2015; Ray et al., 2016; Chen et al., 2015b), and/or different pre-treatments of the waste (Oh et al., 2015; Patel et al., 2015; Kourmentza et al., 2015; Valentino et al., 2015a; Korkakaki et al., 2016; Chen et al., 2015b). Accumulation capacity values seem to not be related to the employment of pure or mixed cultures. Indeed among pure cultures,

cultures of *Cupriavidus necator* were especially relevant due to accumulation capacity values higher than 62%, which were reached in all cases (Schmidt et al., 2016; Cruz et al., 2014; Cruz et al., 2015; Ribeiro et al., 2016; Haas et al., 2015). Additionally, PHA yields strongly varied from 0.02 g PHA/g substrate in the reviewed lab-scale studies (Haas et al., 2015; Alsafadi and Al-Mashaqbeh, 2017) to 0.9 g PHA/g substrate (Colombo et al., 2016; Cruz et al., 2015). Around 61% of the reviewed studies at lab-scale reported values lower than 0.5 g PHA/g substrate (Table 2). Low PHA yield could be closely related to the use of complex waste as a source of carbon, such as olive mill wastewater, i.e. 0.09 g PHA/g substrate (Alsafadi and Al-Mashaqbeh, 2017), chicory roots, i.e. 0.007 g PHA/g substrate (Haas et al., 2015), hydrolyzed effluent from rice bran, i.e. 0.026 g PHA/g substrate (Oh et al., 2015) or pea shells and onion peel slurries 0.04 g PHA/g substrate (Kumar et al., 2016).

In general, values for accumulation capacity and PHA yields were significantly lower at pilot-scale than at lab-scale (Table 2 and Table 3). Most of the pilot-scale studies reached accumulation capacities in the low range of 24 to 35% (Table 3) (Ntaikou et al., 2014; Amulya et al., 2015; Morgan-Sagastume et al., 2015; Chen et al., 2016a). To enhance the accumulation capacity, the addition of an easily degradable additional source of carbon, such as VFA (Jia et al., 2014) or the implementation of pre-treatments to maximize the VFA concentration in the waste have been proposed (Tamis et al., 2014). PHA yields varied from 0.17 g PHA/g substrate to 0.38 g PHA/g substrate (Table 3) (Amulya et al., 2015; Morgan-Sagastume et al., 2015). The low PHA yield at pilot-scale could be explained by the unemployment of synthetic sources of carbon as co-substrates in the PHA accumulation phase, which is widely reported at lab-scale (Kourmentza et al., 2015; Pais et al., 2016; Bera et al., 2015; Korkakaki et al., 2016; Chen et al., 2015b). As an alternative to severe pre-treatments, the selection of most

digestible waste, such as crude glycerol, could enhance the accumulation capacity and PHA yield (Bera et al., 2015; Ray et al., 2016; de Paula et al., 2016).

4. Extraction/Purification Block

The most widely used purification method is a centrifugation, freeze-up, and lyophilisation sequence (Valentino et al., 2015b; Korkakaki et al., 2016; Venkateswar-Reddy et al., 2016). Acetone is used to wash the lyophilized material and chloroform for the extraction. This solution in chloroform eventually precipitates into diethyl ether (Hassan et al., 2013; Liu et al., 2015). Follonier et al. (2015) described a solvent extraction process using methylene chloride from the freeze-dried biomass, and a subsequent purification by precipitation in cold methanol. These methods are intensive in energy and include solvents that are toxic to the environment.

The search for purification steps with lower cost, higher performance, and lower environmental impact than conventional methods is one of the guidelines to be followed in future research. The current purification steps are very energy and solvent intensive and cause losses in PHA. In the specific studies on PHA purification steps, high performance has been achieved with economical and environmentally friendly solvents such as water (Mohammadi et al., 2012).

5. Conclusions

Scaling-up PHA production from waste streams requires further research efforts to become economically attractive, which is the main challenge for the implementation of PHA production from waste streams at industrial scale. Reviewed research studies mainly focus on reducing cost or obtaining more valuable polymers. The selection of an adequate waste stream is critical to ensure an adequate and constant supply. In that sense, wastewater, crude glycerol from the biodiesel industry and whey are gaining

attention as carbon sources. Furthermore, the combination of two or more waste streams could avoid the requirement of additional synthetic streams and/or compensate the nutrients balance in the process. Nowadays, the three-phase process, which includes a pre-treatment phase to adjust the waste material to an appropriate feed stream, is the most commonly employed for PHA production. The integration of PHA production into processes such as wastewater treatment plants, hydrogen production or biodiesel factories could enhance their implementation. In the future, PHA production from waste streams will require flexible processes, which could be adapted to different seasonal waste streams by adjusting operational variables.

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Figure Captions

Figure 1. Number of research studies on PHA production from waste materials in the last 24 years (Scopus, 2017).

Figure 2. Simplified block diagram of the PHA production process by employing waste as a source of organic matter.

Figure 3. Percentage of references per kind of waste. (*AA, Agro-Alimentary wastes; F, Food waste; INAA, Industrial Not Agro-Alimentary waste; UWP, Urban Waste Plan waste; SS, Synthetic Substrates*)

Figure 4. Temporal evolution of the number of references per kind of waste. (*AA, Agro-Alimentary waste; F, Food waste; INAA, Industrial Not Agro-Alimentary waste; UWP, Urban Waste Plan waste; SS, Synthetic Substrates*)

Figure 1.

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graph LR; WCS[Waste carbon source] --> Pretreatment; Pretreatment --> Waste_Energy[Waste/Energy]; Pretreatment --> Production; Production --> Extraction_Purification[Extraction/Purification]; Extraction_Purification --> PHA; Extraction_Purification --> Waste; Extraction_Purification -- "Additional raw materials / Energy" --> Production;
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Figure 3.

Figure 4.

Table 1.Previous reviews on PHA production processes

Authors	Topic	Title	Highlights
Muhammadi et al., 2015	PHA production	Bacterial polyhydroxyalkanoates-eco-friendly next generation plastic: Production, biocompatibility, biodegradation, physical properties and applications	<ul style="list-style-type: none">- Description of PHA production process.- Potential applications according to the biocompatibility of PHA produced.
Anjum et al., 2016	PHA production	Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements	<ul style="list-style-type: none">- Identification of PHA produced industrially: Biopol, Nodax 3HB, DegraPol, Biogreen.- Applications of biopolymers in nanocomposites and mixtures.
Obrucetal, 2015	Use of lignocellulosic materials as substrate	Use of Lignocellulosic Materials for PHA Production	<ul style="list-style-type: none">- Lignocellulosic material is abundant, cheap, and it does not compete with human food chain.- The main challenges are the necessary hydrolysis of the biomass to yield fermentable sugars and presence of numerous antimicrobial agents.
Koller and Murh, 2014	PHA production	Continuous Production Mode as a Viable Process-Engineering Tool for Efficient Poly(hydroxyalkanoate) (PHA) Bio-Production	<ul style="list-style-type: none">- Feasibility study of the process design and operation taking into account: Kinetic particularities, strains, and monomeric composition of the biopolymers.
Koller and Braunegg,	Revalorization of surplus materials to	Biomediated production of structurally diverse poly(hydroxyalkanoates) from	<ul style="list-style-type: none">- Future perspective: the utilization of organic carbon-rich surplus materials from diverse industrial branches and algal oils rich in

2015	PHA production	surplus streams of the animal processing industry	polyunsaturated fatty acids (PUFAs). - Reduce production costs: the integration of PHA-production facilities directly into the production lines.
Mozejko-Ciesielska and Kiewisz, 2016	PHA production	Bacterial polyhydroxyalkanoates: Still fabulous?	- Strategies to optimize PHA production by high-cell-density cultivation. - Develop a PHA overproducing recombinant strain. - Develop a strain that controls the PHA structure generated.
Jian et al., 2016	Biorefinery concept on PHA production	Carbon Sources for Polyhydroxyalkanoates and an Integrated Biorefinery	- Reduce PHA production costs by biorefinery concept.
Wang et al., 2014	PHA production	Polyhydroxyalkanoates, challenges and opportunities	- Strategies to reduce production costs. - Develop a PHA overproducing recombinant strain. - Develop a strain that controls the PHA structure generated.
Wei et al., 2015	Revalorization of PHA production waste by pyrolysis	Production and characterization of bio-oil and biochar from the pyrolysis of residual bacterial biomass from a polyhydroxyalkanoate production process	- Reduce PHA production costs in continuous processes. - Re-use the bacterial biomass generated in the PHA production for the production of biofuel and biochar by a pyrolytic step.
Chen et al., 2015a	Mechanisms of polymer	Engineering Biosynthesis Mechanisms for Diversifying	- Study of the three-dimensional structure of PHA. - Generate different types of polymers.

	diversification	Polyhydroxyalkanoates	- Increase diversity of materials produced from PHA.
Kaur and Roy, 2015	PHA production	Strategies for Large-scale Production of Polyhydroxyalkanoates	<ul style="list-style-type: none"> - Reduce the PHA production costs. - Increase the value of the products obtained from PHA. - Implement the process industrially.
Liu et al., 2015	PHA production	Recent strategies for efficient production of polyhydroxyalkanoates by micro-organisms	<ul style="list-style-type: none"> - Obtain renewable carbon sources for PHA production. - Optimize genetic modification of the strain. - Optimize pretreatment and purification. - Reduce energy consumption of the process. - Potentiate use of precursors in the PHA production.
Kawaguchi et al., 2016	Polymer production from lignocellulosic matter	Bioprocessing of bio-based chemicals produced from lignocellulosic feedstocks	<ul style="list-style-type: none"> - The yield of lignocellulosic matter to PHA production. - Increase enzymatic activity. - Improve extraction. - Identify inhibiting factors in PHA production.
Tai et al., 2016	PHA production	Discovery of a new polyhydroxyalkanoate synthase from limestone soil through metagenomic approach	<ul style="list-style-type: none"> - Genetic studies to increase PHA productivity.
Inoue et al., 2016	PHA production	Polyhydroxyalkanoate production potential of heterotrophic bacteria in activated sludge	<ul style="list-style-type: none"> - DNA analysis. - Qualitative evaluation of PHA production from different bacteria. - Correlation between the carbon source and genetic sequences of PHA production.

Valentino et al., 2017	PHA production	Carbon recovery from wastewater through bioconversion into biodegradable polymers	<ul style="list-style-type: none"> - PHA production price decrease from € 10-12 / kg to € 2.2 / € 5 / kg. - Description of the production scheme based on three stages: acidification, biomass growth, and PHA accumulation. - Integration of PHA production into the wastewater treatment plants.
Koller et al., 2017	PHA production	Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner	<ul style="list-style-type: none"> - Possible types of feed streams to reduce costs: biodiesel by-products, whey, food by-products and lignocellulosic crops. - Integration of the PHA process into biodiesel production plants.
Mohan et al., 2016	Waste biorefinery	Waste biorefinery models towards sustainable circular bioeconomy: Critical review and future perspectives	<ul style="list-style-type: none"> - Implementation of PHA production into biorefineries based on CO₂ capture and holistic biorefineries.
Zia et al., 2016	PHA production	Recent developments and future prospects on bio-based polyesters derived from renewable resources: A review	<ul style="list-style-type: none"> - Potential applications of PHA produced.

Table 2. Reviewed research articles at lab-scale.

Reference	Culture	Employed substrate	Pre-treatment	Additional stream	Operation mode	Reactor size (L)	Waste Yield	Substrate yield	Accumulation capacity %
Pittmann and Steinmetz, 2016	Mixed culture: active sludge	Activated wastewater sludge (theoretical study)	Solid/liquid separation; anaerobic phase (hydrolysis); dilution water;	Water (dilution) Nutrients	3 Phases: Pre-treatment; Biomass accumulation and PHA production. Batch operation.	Theoretical model	0.26 g PHA/g sewage sludge	1.18 g PHA/g substrate	28.4
Schmidt et al., 2016	Pure culture: <i>C. necator</i>	Synthetic waste (glucose)		Propionic acid Synthetic medium	2 Phases: Growth and PHA production. Batch operation.	5	Synthetic	0.26 g PHA/g Substrate	73
Gómez-Cardozo et al., 2016	Pure culture: <i>Bacillus megaterium</i> , <i>Bacillus sp.</i> , and <i>Lactococcus lactis</i>	Cheese whey Used cooking oil Crude glycerol		Synthetic medium HCl / NaOH	1 Phase: Growth and PHA production. Sequential operation.	0.05	No data	0.2 g PHA/g Substrate	87
Cruz et al., 2016	Pure culture: <i>C. necator</i>	Olive oil distillate Used cooking oil biodiesel fatty acids-by-product		Synthetic medium	1 Phase: PHA production. Batch operation.	0.25	0.27 g PHA/g COD	0.9 g PHA/g substrate	62
Oh et al., 2015	Pure culture: <i>E. coli</i> and <i>R. eutropha</i> (Recombinants)	rice bran	Hydrolysis; ultra filtration; and reverse osmotic;sterilized	Synthetic medium NH ₄ OH Antifoam	3 Phases: Pre-treatment, Growth, PHA production. Batch operation.	1	0.003 g PHA /g Rice bran	0.026 g PHA /g substrate	97.2
Patel et al., 2015	Pure culture: <i>B. cereus</i>	pea-shell	Centrifugation; pH adjustment	Glucose Synthetic	3 Phases: Pre-treatment; Growth and	0.2	No data	0.35 g PHA/g substrate	64.7

EGU43				medium	PHA production. Batch operation.				
Kumar et al., 2016	Pure culture: <i>Bacillus</i> ssp	pea-shells potato peels apple pomace onion peels (mixtures)	Hydrolysis, solid/liquid separation; filtration;	Glucose Synthetic medium	3 Phases: Pre-treatment; Culture growth and PHA production. Sequential operation.	0.2	No data	0.04 g PHA/g substrate	7.5
Bhati and Mallick, 2016	Pure culture: <i>Cyanobacterias</i> <i>Nostoc muscorum</i>	poultry litter+ CO ₂ -enriched air (synthetic flue gas)	Dried under sunlight; Mixed distilled water; Filtered	Synthetic medium Acetate, glucose and valerate	2 Phase: Pre-treatment and Growth / PHA production. Sequential operation.	4	No data	0.08 g PHA/g substrate	65
Alsafadi and Al-Mashaqbeh, 2017	Pure culture: <i>H. mediterranei</i>	Olive mill wastewater	Dilution in nutrient limited medium	Synthetic medium	3 Phases: Pre-treatment; Growth and PHA production. Batch operation.	0.1	0.001 g PHA/g OMW	0.009 g PHA/g VFA	43
Kourmentza et al., 2015	Mixed culture	Olive mill wastewater	Water dilution; Anaerobic digestion (mesophilic); Centrifugation; filtration; phosphate buffer solution; sterilization/ solid phase extraction (dephenolized wastewater); mesophilic anaerobic acidogenic packed-bed biofilm reactor	Water (dilution) Synthetic medium phosphate buffer solution	3 Phases: Pretreatment; Enrichment under stress conditions and PHA production. Batch operation.	2.4	No data	0.18/0.68 g PHA/g substrate	64.4/18.2
Campanari et al., 2014	Mixed culture: activated sludge	olive mil wastewater		Synthetic medium CO ₂ (flushing)	3 Phases: Pre-treatment; Enrichment (Feast/famine) and PHA production. Batch operation.	0.35	0.57 g PHA/g OMW	0.86 g PHA/g substrate	30

				(increase volatile fatty acids concentration)					
Elain et al., 2016	Pure culture: <i>Halomonas H. i4786</i>	Leguminous Processing Wastewater Fruit Processing Wastewater		Synthetic medium HCl / NaOH Antifoam	2 Phases: Growth and PHA production. Batch operation.	2	No data	0.17/0.13 g PHA/g substrate	55/78
Valentino et al., 2015a	Mixed culture	Cheese whey	Centrifugation	Synthetic medium NH ₄ Cl and/or KH ₂ PO ₄	3 Phases: Pre-treatment; feast-famine regime and PHA production. Batch operation.	0.5	No data	0.28/0.3 g PHA/g substrate	58/75
Valentino et al., 2015b	Mixed culture	Cheese whey	Anaerobic batch fermentation; Centrifugation	Synthetic medium NH ₄ Cl and/or KH ₂ PO ₄	3 Phases: Pre-treatment; Growth and PHA production. Batchoperation.	0.5	No data	0.46 g PHA/g substrate	No data
Pais et al., 2016	Pure culture: <i>H. mediterranei</i>	Cheese whey	Acid hydrolysis	Synthetic medium HCl / NaOH Antifoam	2 Phases: Pre-treatment and PHA production. Sequential operation.	2	0.49 g PHA/g Cheese whey	0.78 g PHA/g substrate	53
Colombo et al., 2016	Mixed culture: Activated sludge	Cheese whey	Fermentation and sterilized fermentation	Synthetic medium Allythiourea	3 Phases: Pre-treatment, Enrichment (feast/famine) and PHA production. Batch operacion.	0.5	No data	0.9 mmolC PHA/mmol C substrate	39
Cruz et al., 2014	Pure culture: <i>Cupriavidus necator</i> DS M 428	spent coffee grounds	Supercritical CO ₂ extraction	Synthetic medium NaOH	2 Phase: Pre-treatment and Growth / PHA production. Sequential	1.5	No data	0.65 g PHA/g substrate	78

					operation.				
Cruz et al., 2015	Pure culture: <i>Pseudomonas necator</i> DSM 428	used cooking oil		Synthetic medium NaOH	2 Phases: Growth and PHA production. Sequential operation.	2	0.37 g used cooking oil/g PHA	0.41 g substrate (VFA)/g PHA	63
Bera et al., 2015	Pure culture: <i>Halomonas hydrothermalis</i>	crude glycerol +oil cake hydrolysate	hydrolysate	synthetic medium Seaweed derived crude levulinic acid	3 Phases: Pretreatment, Growth and PHA production. Batch operation.	0.1	No data	0.75 g PHA/g substrate	73.3
Ribeiro et al., 2016	Pure culture: <i>Cneçator</i> and <i>B. megaterium</i>	crude glicerol		Synthetic medium HCl / NaOH	2 Phases: Growth and PHA production. Sequential operation.	0.052	No data	0.23 g PHA/g Substrate	67
Ray et al., 2016	Pure culture: <i>Pannonibac terphragmitetus</i> ERC8	Crude glycerol	Dilution	Synthetic medium pH Control*	3 Phases: Pretreatment, Isolation and enrichment and, PHA production. Batch operation.	3	No data	0.16 g PHA/g substrate	64.34
Haasetal, 2015	Pure culture: <i>C. necator</i> DSM428, 531 and 545	ChicoryRoots	Dried,milled, hydrolysis, detoxification, centrifugation and filtration	Synthetic medium HCl/NaOH	3Phases: Pretreatment, Growth and PHA production. Batch operation.	1	No data	0.02/0.01/0.03 g PHA/g Substrate	66/46/78
de Paula et al., 2016	Pandoraea sp.	Crude glycerol		Synthetic medium Propionic acidol	2Phases: Isolation and Growth and, PHA production. Batch operation.	0.1	No data	0.16/0.22/0.05/0.05/0.04 gPHA/g Substrate	37/49/12/12/10

Hexanoic acid									
Pan et al., 2016	Pure culture: <i>Corynebacterium hydrocarboxydans</i> and <i>Bacillus megaterium</i>	Carboxylic acid (in glycerol fermentation effluent from propanediol manufacturing)	Anaerobic fermentation	Synthetic medium	3 Phases: Pre-treatment; Growth and PHA production. Batch operation.	10	0.01 g PHA/g glycerol	0.02 g PHA/g Substrate	21.4
Cha et al., 2016	Mixed culture: active sludge	Activated wastewater sludge		Sodium acetate (first substrate) Nutrients (N/P) and trace elements	2 Phases: Acclimatization anaerobic/aerobic and PHA production. Sequential operation.	2	No data	No data	63
Korkakaki et al., 2016	Mixed culture: activated sludge	Municipal solid waste leachate	Acid fermentation; hydrolyzed in acidification tunnels; phosphate removal (Aeration)	Synthetic medium Phosphate VFAs solution (first substrate)	3 Phases: Pretreatment, enrichment (feast/famine) and PHA production. Batch operation.	2	0.3 g PHA/g raw leachate	0.38/0.47/0.66/0.62 g PHA/g substrate	n/n/n/78/77
Basset et al., 2016	Mixed culture: Activated sludge	Urban wastewater+ Organic fraction of municipal solid waste	Fermentation; solid/liquid separation	-	3 Phases: Pre-treatment, Enrichment and PHA production. Batch operation.	0.8	No data	0.22 g PHA/g Substrate	11
Rodriguez-Contreras et al., 2016	Pure culture: <i>Bacillus megaterium</i> <i>uyuni S29</i>	Synthetic waste (glucose+ high NaCl)		Synthetic medium	2 Phases: Growth and PHA production. Batch operation.	0.1	No data	0.22 g PHA/g substrate	22.5
Palmeiro-	Mixed	Synthetic waste		Synthetic	2 Phases:	1.8	Synthetic	0.172 Cmol	53

Sánchez et al., 2016	culture: active sludge	(VFAs+ high NaCl)		medium	Enrichment culture (feast-famine regime); and PHA production. Batch operation.			HB/Cmol VFA 0.142 Cmol HV/Cmol VFA	
Amulya et al., 2016	Mixed culture: active sludge	Synthetic waste (glucose)		Synthetic medium	2 Phases: Enrichment culture (feast-famine) and PHA production. Sequential operation.	0.11	No data	0.35 g PHA/g substrate	56
Venkateswar-Reddy et al., 2015	Pure culture: <i>Bacillus CYRI</i>	Synthetic waste (toxic compounds)		Tween-80	2 Phases: Growth and PHA production. Batch operation.	0.1	0.1/0.076 g PHA/g phenol/ naphthalene	0.01/0.076 g PHA/g substrate	51/42
Chen et al., 2015b	Mixed culture: activated sludge	Synthetic waste (VFAs)	Fermentation	Synthetic medium	3 Phases: Pretreatment; Enrichment (feast/famine) and PHA operation. Batch operation.	1.4/1	No data	0.81/0.4 C mol PHA/ C mol VFA	70.4/60
Chen et al., 2016a	Mixed culture: active sludge	Synthetic waste (VFAs)		Synthetic medium	2 Phases: feast-famine and PHA production. Batch operation.	0.5	No data	2.3 maximum modeled biomass specific PHA production rate (Cmmol/Cmmol /h)	No data
Venkateswar-Reddy et al., 2016	Pure culture Pure culture: <i>H.palleronii</i>	Synthetic waste (VFAs)		Synthetic medium	2 Phases: Culture grow and PHA production. Batch operation.	0.1	Synthetic	0.05 g PHA/g Substrate	63
Fradinho et	Mixed	Synthetic waste		Synthetic	1 Phase: Growth	0.45	No data	0.67 g PHA/g	45

al., 2016	culture: Activated sludge and algae	(VFAs)	medium	in feast/famine regime and PHA production. Sequential operation.			Substrate	
Kaewbai- ngam et al., 2016	Pure culture: <i>Calothrixsc ytonemicola</i>	Synthetic waste (pure CO ₂)	Synthetic medium	1 Phases: Growth and PHA production Sequential operation.	0.1	No data	0.5 g PHA/g Substrate	25.4

* *Not specified chemical*

Table 3. Reviewed research articles at pilot-scale.

Reference	Culture	Employed substrate	Pre-treatment	Additional stream	Operation mode	Reactor size (L)	Yield	Accumulation capacity (%)
Ntaikou et al., 2014	Mixed culture // Pure culture: <i>Pseudomonas sp.</i>	olive-mill wastewater	Acidification; Water dilution; Floculation: aluminium sulphate	Waster (dilution) Nutrient solution HCl and NaOH butyrate and acetate (first substrate)	3 Phases: Pre-treatment, enrichment (acclimation) and PHA production. Continuous operation.	30	7.58 ± 0.06 g PHAs per L of initial OMW 0.00758 g/g	25
Tamis et al., 2014	Pure culture: <i>Plasticic umulans acidivorans</i>	Industrial Wastewater (candy bar factory)	Hydrolysis (Anaerobic fermentation)	Waster (dilution) Nutrients solution (including trace elements) HCl and NaOH Allythiurea	3 Phases: Pre-treatment, enrichment (feast/famine) and PHA production. Continuous operation.	200	0.37 g PHA/g substrate	70
Amulya et al., 2015	Mixed culture: activated sludge	Composite food waste: boiled rice, cooked vegetables, un-cooked vegetables	Masticated; filtered; gravity separator; diluted; pH adjustment);	Nutrients solution pH Control*	3 Phases: Pre-treatment, enrichment (feast/famine) and PHA production.	20	0.17 g PHA/g substrate	24

		(spoiled), cooking oil, vegetable peelings, cooked meat, boiled spices	Hydrolysis (Acidogenic fermentation)	Urban wastewater	Continuous operation.			
Morgan-Sagastume et al., 2015	Mixed culture: active sludge	Activated wastewater sludge	Hydrolysis; Centrifugation and filtration	Urban wastewater	3 Phases: Pre-treatment, enrichment (feast/famine) and PHA production. Continuous operation.	400	0.38 g PHA/g substrate	35
Jia et al., 2014	Mixed culture: active sludge	Activated wastewater sludge	Hydrolysis (Anaerobic fermentation); filtration and dilution	Synthetic medium (including VFAs)	3 Phases: Pre-treatment, enrichment (feast/famine) and PHA production. Continuous operation.	70	0.17 g PHA/g substrate	59
Follonier et al., 2015	Pure culture: <i>Pseudomonas putida</i> KT2440	Grape pomace	Water extraction, centrifugation and filtration	Octanoic acid and 10-undecenoic acid	3 Phases: Pre-treatment, enrichment (acclimation) and PHA production. Feed-batch mode.	100	0.015 g PHA/g substrate	41.1

** Not specified chemical*