Waste Management 119 (2021) 374–388

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Waste to bioplastics: How close are we to sustainable polyhydroxyalkanoates production?

Kasra Khatami, Mariel Perez-Zabaleta, Isaac Owusu-Agyeman, Zeynep Cetecioglu *

Department of Chemical Engineering, KTH Royal Institute of Technology, SE-100 44 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 3 August 2020
Revised 30 September 2020
Accepted 2 October 2020
Available online 01 November 2020

Keywords:
Polyhydroxyalkanoates
Waste streams
Bioproduction
Metabolic engineering
Bioaugmentation

ABSTRACT

Increased awareness of environmental sustainability with associated strict environmental regulations has incentivized the pursuit of novel materials to replace conventional petroleum-derived plastics. Polyhydroxyalkanoates (PHAs) are appealing intracellular biopolymers and have drawn significant attention as a viable alternative to petrochemical based plastics not only due to their comparable physiochemical properties but also, their outstanding characteristics such as biodegradability and biocompatibility. This review provides a comprehensive overview of the recent developments on the involved PHA producer microorganisms, production process from different waste streams by both pure and mixed microbial cultures (MMCs). Bio-based PHA production, particularly using cheap carbon sources with MMCs, is getting more attention. The main bottlenecks are the low production yield and the inconsistency of the biopolymers. Bioaugmentation and metabolic engineering together with cost effective downstream processing are promising approaches to overcome the hurdles of commercial PHA production from waste streams.

Contents

* Corresponding author.
E-mail address: zeynepcg@kth.se (Z. Cetecioglu).

https://doi.org/10.1016/j.wasman.2020.10.008
0956-053X/

1. Introduction

Burgeoning concerns about rapid depletion of fossil fuel resources as well as adverse environmental impacts of conventional petroleum-based plastics are the driving force of the research pursuing materials production from sustainable resources and green processes. Plastics have become an indispensable commodity in today's life due to their wide range of applications, particularly in packaging (Groh et al., 2019). Plastics Europe (2019), reported that global manufacturing of plastics reached 348 million tonnes in 2017, that was 4% increment compared to the previous year. Disposal methods of plastic materials such as landfilling and incineration are associated with high costs and creation of toxic byproducts. Petroleum-derived plastics have low biodegradability and their recycling processes are tremendously slow and time consuming (Anjum et al., 2016). As a result, only 9% and 26% of post-consumer polymers are recycled in the United States and Europe, respectively (Dietrich et al., 2017).

Polyhydroxyalkanoates (PHAs) are microbiologically produced polyesters with tunable mechanical and physical properties. PHAs have high biodegradability and biocompatibility and therefore they are environmentally friendly (Dietrich et al., 2017). According to Hassan et al. (2013) the required time for their biodegradation to CO_2, water and biomass (Chen, 2010), under standard conditions is approximately two months, which significantly diminishes the environmental impact of plastic wastes. PHAs have a broad range of applications in numerous sectors namely, packaging, biomedical, agriculture, production of latex paints (Muhammadi et al., 2015), biofuels (blended with diesel or gasoline) and building block of various biochemicals (Chen, 2009). The aforementioned characteristics make PHAs a promising bioplastic alternative to conventional petrochemical plastics.

Microbial PHAs were discovered in the 1920s when intracellular granules of the biopolymer, poly-3-hydroxybutyrate (PHB), were observed in the bacterium *Bacillus megaterium* (Lemoigne, 1926). Microorganisms can accumulate PHA up to 97% of cell dry weight (CDW) (Muhammadi et al., 2015) in their cytoplasm and use it as energy storage. Surplus quantities of carbon source with limitation of other crucial nutrients e.g. nitrogen or phosphorous and sometimes with pH shifts are the required conditions to stimulate the PHA accumulation in bacterial cytoplasm (Koller et al., 2008; Shah et al., 2008). A Diverse variety of microorganisms possess the ability to synthesize PHA from a broad range of renewable resources. More than 300 species, including bacteria (both gram-negative and gram-positive) (Anjum et al., 2016) and archaea (Hermann-Krauss et al., 2013), have been identified to accumulate PHAs (Zinn et al., 2001) under both aerobic and anaerobic conditions (Kim et al., 2007).

Commercial bio-based production of PHAs has been realized only to a limited scale. Countries such as Germany (Biomer) and Italy (Bio-on) are producing PHAs using expensive feedstocks such as sugars which their usage and sustainability is currently debatable. Production costs and market price are deemed as the two main drivers that could shift the plastic production from petrochemicals to bio-based (Kourmentza et al., 2017). Carbon source and the extraction of biopolymers account for 40–48% and 30% of the total production costs, respectively (Sun et al., 2007). The market price of PHA polymers is approximately €5 per kg, which is almost 6 times higher compared to petroleum-based (€0.8–1.5 per kg) (Oever et al., 2017). Nonetheless, due to environmental awareness and the subsequent legislations on prohibition or reduction of plastic materials such as the European Parliament's Directive on single use plastics (EUPPD, 2019), the production of bioplastic in general; and PHAs in particular, is rapidly increasing. The global bioplastics production capacity was 2.11 million tonnes in 2018 and is expected to exceed 2.6 million tonnes in 2023 (European Bioplastics, 2018). The global PHA market was valued at US$73.6 million and is projected to reach US$93.5 million by 2021 with a compound annual growth rate (CAGR) of 4.88% (Sun et al., 2007).

As mentioned above, there are still bottlenecks for realization of industrial scale PHA production. The objective of the present review is not only to outline the basics of PHAs and the current status of the production from both pure and mixed microbial cultures, but also to present suggestions as a blueprint for future direction of the research work on utilization of waste streams as cheap viable substrates, metabolic engineering and bioaugmentation strategies to increase the production efficiency and greener PHA recovery techniques.

2. Structure and properties of PHA

PHAs have drawn much attention owing to their similarities to petrochemical polymers such as polypropylene and polystyrene (Sudesh et al., 2000), which make them a sustainable alternative to replace a substantial number of petroleum-based plastics that are used in diverse applications. PHAs are bio-polymers, synthesized inside the cellular structure of microorganisms in granular shapes with diameters ranging between 0.2 and 0.5 μm (Raza et al., 2018). They can be dissolved in chlorinated solvents (Raza et al., 2018). Features of PHA can differ from brittle thermoplastics to gummy elastomers (Dietrich et al., 2017) depending on the type of the utilized carbon source, bacterial host (Możejko-Ciesielska and Kiewisz, 2016) and fermentation conditions (Anjum et al., 2016). The composition and structure of the biopolymers determine their degradation in the environment (Emadian et al., 2017).

The categorization of PHAs, is according to the number of the present carbon atoms in their constituent monomer blocks. Short chain length (scl-PHAs) and medium chain length (mcl-PHAs), comprising of 3 to 5 and 6 to 14 carbon atoms, respectively (Raza et al., 2018; Albuquerque and Malafaia, 2018; Muhammadi et al., 2015). Different chain lengths originates from the substrate specificity of the PHA synthase enzyme that is capable of accepting a 3-hydroxyalkanoic acid containing a particular number of carbons (Khanna and Srivastava, 2005). Scl-PHAs are comparatively brittle and stiff because of their crystalline structure (typically 55–80% crystallinity). These relatively poor properties could explain why several efforts are made to modify and enhance the properties of scl-PHAs (Wang et al., 2016). In contrast, mcl-PHAs are rubbery and elastic materials (Madison and Huisman, 1999). Till today, the number of the discovered PHA monomer constituents exceeds 150 and novel monomer compositions are still getting identified particularly; by using recombinant species (Sharma et al., 2017). It is therefore evident that if given the needed attention, PHAs can replace all fossil-based plastics.

3. Microbial cell factories for PHA production

3.1. Pure cultures

PHA producing microorganisms encompass a broad range of gram-positive and gram-negative bacteria such as *Cupriavidus necator* (accumulation up to 90% cell dry weight (CDW)) (Passanha et al., 2013), *Bacillus megaterium* (accumulation up to 62% CDW) (Faccin et al., 2013) and different archaea species from the family *Halobacteriaceae* (e.g. *Haloferax* and *Haloarcula*) have been also identified as PHA producers (Han et al., 2010; Hermann-Krauss et al., 2013; Taran et al., 2011).

There are two distinct classifications of bacteria, regarding PHA synthesis. The first group requires stress conditions such as limitation or depletion of crucial nutrient (e.g. nitrogen, phosphorous) while the carbon source is present in excess. *Cupriavidus necator* (also known as *Ralstonia eutropha*) and *Pseudomonas oleovorans* belong to this group (Lageveen et al., 1988; Schlegel et al., 1961). On the other hand, nutrient starvation is not required by the second group and PHAs can be accumulated during the growth phase. Bacteria that belong to this group are recombinant *Escherichia coli*, *Alcaligenes latus*, a mutant strain of *Azotobacter vinelandii* and *Actinobacillus* sp. EL-9 (Hänggi, 1990; Page and Knosp, 1989; Son et al., 1996).

3.1.1. Metabolic engineering

Metabolic engineering and classical-strain-improved techniques have been extensively implemented to manipulate and improve PHAs production with the main focus of making them more market-competitive. PHA-producer microorganisms have been improved for several decades and some of the earliest examples date back to the use of random mutagenesis by exposing microbial cells to chemicals or UV light. As an example, *C. necator* was chemical-mutated to increase the production fraction of (*R*)-3-hydroxyvalerate in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P (3HB-co-3HV)] (Lee et al., 1996). Adaptive evolution, a classical-strain-improved technique, has been also widely used to increase PHA accumulation in microorganisms e.g. an evolved *C. necator* for glycerol assimilation produced 19% more PHB and grew 9.5 times more rapid compared to the wild-type strain (González-Villanueva et al., 2019). Nowadays, identifying the genetics modifications that improved the cell function by random mutagenesis or adaptive evolution is of high importance to understand the new phenotype obtained. Reintroducing these mutations into the parental strain results in reverse engineering of the metabolic pathways. By reverse engineering, it has been shown that YfcX (3-hydroxyacyl-CoA dehydrogenase, a subunit of fatty acid oxidation complex) is crucial in the synthesis of mcl-PHAs (Snell et al., 2002).

Polyhydroxyalkanoates are considered model compounds for metabolic engineering due to the wide diversity of PHA-monomers and because their pathways are well-studied (Agnew and Pfleger, 2013). Metabolic engineering has been applied into PHA-producers to improve cultivation parameters such as yield, concentration and production rate by (1) expanding substrate utilization, (2) engineering cells morphology, (3) pathway optimization, elimination of competitive pathways/reactions, (4) increasing precursor availability and (4) cofactor supply and regeneration. Additionally, metabolic engineering has been also used to enhance the quality of the PHAs by controlling e.g. chain length, monomeric composition and molecular weight.

An ideal PHA producer should have a high growth rate, in which metabolic engineering can be used to accelerate growth (Ren et al., 2018). A high growth rate is directly related to fast carbon source consumption, which is of paramount significance when waste streams such as lignocellulose or volatile fatty acids (VFAs) are utilized. Metabolic strategies to enhance the substrate utilization depends on the carbon source e.g. simultaneous consumption of glucose, xylose and arabinose (the dominant sugars present in lignocellulose) during production of (*R*)-3-hydroxybutyrate, monomer of PHB, was achieved by deleting *ptsG* gene and evolving the recombinant strain in arabinose (Jarmander et al., 2015).

In addition to the number of produced cells per time and fast carbon source consumption, engineering the morphology of cells is also an important factor to improve PHA accumulation (Jiang and Chen, 2016) e.g. *Halomonas* TD01 was engineered using CRISPRi, in which the expression of FtsZ was repressed resulting in elongated and more filamentous cells that can harbor higher amount of PHA (Tao et al., 2017). Cell morphology of *E. coli*, a recombinant PHA-producer, was also modified by overexpressing *sulA* gene and resulted in more than 100% increase of PHB content (Wang et al., 2014a).

Optimization of the metabolic pathways (Fig. 1) is a priority, where the expression of PHA-enzymes should be sufficiently high to allow a rapid synthesis of the polymer, while being carefully balanced to avoid the formation of intermediate products or causing metabolic burden to the cell (Lee et al., 2012). A more optimal pathway can be obtained by engineering the promoter strength

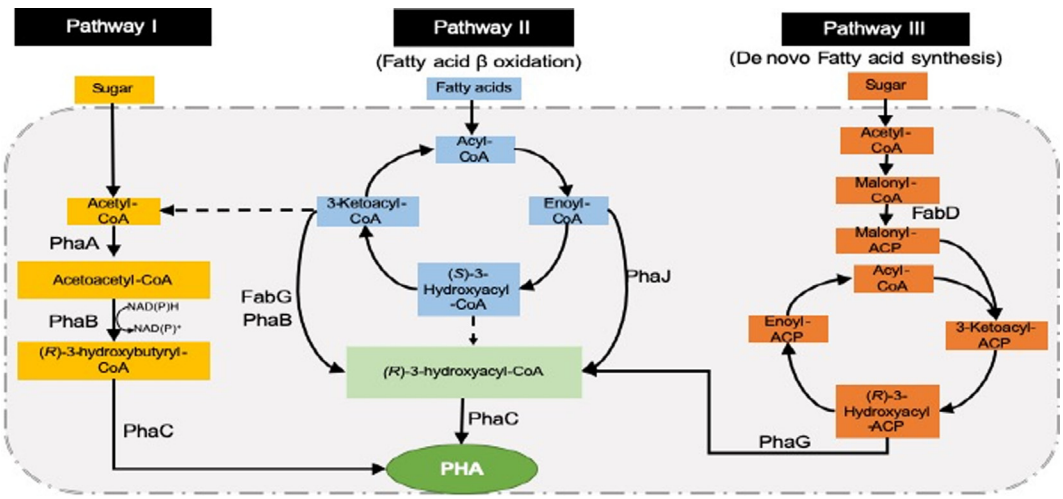

Fig. 1. The 3 main metabolic pathways for PHA biosynthesis. PhaA is β-ketothiolase; PhaB is acetoacetyl coenzyme A(CoA) reductase: PhaC is PHA synthase; FabG is 3-ketoacyl acyl carrier protein (ACP) reductase; PhaG is acyl-ACP-CoA transacylase; PhaJ is enoyl-C.

Table 1
Comparison of PHA production by pure cultures in laboratory scale.

Strains	Carbon Source	Scale	Types of PHA	pH	PHA content (%)	Productivity or yield	References
Cupriavidus necator	Cheese whey (Concentrated with an electrodialysis step)	3 L	3HB 3HV	7	71%	0.6 g PHA/g VFA	Domingos et al., 2018
Cupriavidus necator	Waste rapeseed oil	2 L	3HB 3HV	7	76%	0.83 g PHA/g oil	Obruca et al., 2010
Bacillus megaterium	Sucrose	5 L	3HB	No control	62%	0.32 ± 0.004 g PHA/g sucrose	Faccin et al., 2013
Pandoraea sp. MA03	Crude glycerol	250 ml	3HB	7	49%	0.22 g PHA/ g crude glycerol	de Paula et al., 2017
Recombinant Cupriavidus necator	Fructose + canola oil	50 ml	3HB, 3HV, 3HHX[a], 3HO[b]		96%		Valdés et al., 2018
Pseudomonas aeruginosa ATCC 27,853	Long odd chain fatty acids (heptadecanoic acid, nonadecanoic acid, heneicosanoic acid)	2 L	–	7	13.4%	196 mg/l	Impallomeni et al., 2018
Pseudomonas putida	Glucose + glycerol + octanoate	1 L	HHx, HO, HD		57%		Fontaine et al., 2017
Recombinant pseudomonas putida	Waste vegetable oil	100 ml	HHx, HO, HD	6.9	38.3 ± 3.1%	0.62 ± 0.1 g PHA/ g oil	Borrero-de Acuña et al., 2019
Burkholderia sacchari	Waste paper		PHB	7.2	44.2%	0.15 g PHA/ g sugar	Al-Battashi et al., 2019
Burkholderia sacchari	Glucose + different co-substrates	2 L	PHB PHV	7	2.7 – 73.7%		Mendonça et al., 2014
Bacillus sp. ISTVK1	Pure glycerol	NA	PHV	7.6	85.19%		Morya et al., 2018

[a] 3-hydroxyhexanoate
[b] 3-hydroxyoctanoate

of the enzymes (Shen et al., 2018), increasing the enzyme activity by codon optimization or site-directed mutagenesis (Zhang et al., 2019), by heterologous expression of enzymes from different microorganisms or by eliminating competitive pathways that lower the PHA contents. One prominent competitive reaction in the PHA pathway is the polymer degradation which is a result of endogenous PHA depolymerase (*phaZ*) and it has been shown that deletion of this gene in *P. putida* improved the accumulation of PHA from 66 to 86 wt% of its CDW (Cai et al., 2009). Recombinant production of PHA resulted in an approach to avoid PHA depolymerization since model microorganisms as *E. coli* lack *phaZ* gene. However, acetate formation in *E. coli* is the main competitive pathway during the production of PHAs or their monomers, which has led to several studies showing metabolic strategies to overcome it and thus increase titer, yield and rate of the product (Perez-Zabaleta et al., 2019; Zhuang and Qi, 2019). Perez-Zabaleta et al., (2019) obtained the titer to highest volumetric productivity of (*R*)-3-hydroxybutyrate (1.52 g $L^{-1}h^{-1}$) and increased 2.3-fold the final titer by using a recombinant *E. coli* with low acetate production.

In addition to pathway optimization and elimination of competitive pathways/reactions, another approach for redirecting flux toward product is to increase precursor availability, which in most of the PHAs pathways the main precursor is acetyl-CoA (Fig. 1). Availability of acetyl-CoA can be increased by overexpressing genes involved in its formation or by the design of new pathways such as the case of SACA (Synthetic Acetyl-CoA) pathway. SACA can provide acetyl-CoA from one-carbon source, it has three reaction-steps and it is a carbon conserved and ATP-independent pathway (Lu et al., 2019). Another study showed that acetyl-CoA availability was increased 6-fold by overexpressing phosphoketolase, which catalyzes the phosphorolysis of both fructose-6-phosphate and xylulose-5-phosphate into glyceraldehyde-3-phosphate and acetyl-phosphate (Anfelt et al., 2015). These are two examples of metabolic engineering that can be applied in natural or non-natural producers to enhance PHA synthesis.

Cofactor availabilty and regeneration is an essential point to consider during PHA production since the second step catalyzed by acetoacetate-reductase (PhaB) requires NAD(P)H as a cofactor (Pathway I, Fig. 1). Most of the PHA-producer microorganisms use NADPH as a cofactor, only *Chromatium vinosum* reductase was found to be NADH-dependent (Liebergesell and Steinbuchel, 1992) and *Halomonas boliviensis* reductase can use both cofactors but with a preference for NADPH (Perez-Zabaleta et al., 2016). Improving NADPH availability by overexpressing glucose-6-phos phate-dehydrogenase (*zwf*) resulted in 41% higher PHB (LIM et al., 2002) and in 50% higher (*R*)-3-hydroxybutyrate titers (Perez-Zabaleta et al., 2016). The yield of PHB over glucose was increased by 76% when NADPH supply was increased by the over-expression of NAD-kinase (Li et al., 2009). Alternatively, insertion of phosphoribulokinase and ribulose-1,5- bisphosphate carboxylase/oxygenase (RuBisCo) in the PHA pathway might enable re-use of excess NADH and increasing product yield on sugar.

Co-production of one or more products in addition to PHA is also another approach to decrease production cost. For example, metabolically engineered *E. coli* produced succinate (21.07 g L^{-1}) and PHA (0.54 g L^{-1}) from a mixture of glycerol, glucose and fatty acids through deletion of *ptsG*, *sdhA* and *pta* genes and overexpression of phaC1 from *P. aeruginosa* (Kang et al., 2011). Co-production of ectoine and PHB by *H. boliviensis* were also proposed as an approach to maximize yield and decrease production cost (Guzmán et al., 2009).

The large number of metabolic engineering studies published on PHA production demonstrates the value of PHAs as commercially viable products and many more significant achievements in the field are expected in the near future.

Table 2
Comparison of PHA production by mixed cultures in laboratory and pilot scale.

Strains	Carbon Source	Scale	Types of PHA	pH	PHA content (%)	Productivity or yield	References
Anerobic sludge + aerobic secondary sludge	Crude glycerol	1.7 L	3HB 3HV 3HP	8		0.42 Cmol PHA/Cmol substrate	Burniol-Figols et al., 2018
Activated sludge	Yeast production wastewater	2 L	PHB PHV	____	65–72%		Bhalerao et al., 2020
Activated sludge	Cheese whey (deproteinized)	0.5 L		____	62 ± 4.5%	0.77 ± 0.14 g COD_{PHA}/g $COD_{organic}COD_{acids}$	Colombo et al., 2019
Activated sludge + bioaugmentation	Sodium acetate	2 L	PHB	7	85%		Marang et al., 2018
Activated sludge	Acetate	2 L	PHB	7	89%	0.43 g PHA/g HAc	Johnson et al., 2009
Activated sludge	Acetic, propionic, butyric and valeric acids	2 L	PHB PHV	7–8	69%	1 ± 0.11 Cmol PHA/Cmol VFA	Wang et al., 2017
Activated sludge	Fermented primary sludge + fermented carbohydrate rich effluent of candy factory + acetic and propionic acids	500 L	PHBV	____	37 ± 5%	0.41 g PHA/gVSS	Werker et al., 2018
Activated sludge	Organic fraction of municipal solid waste + sewage sludge	50–70 L	PHB PHV	____	46%	65 g PHA/ kg TVS	Valentino et al., 2019
Activated sludge	Acetate	550 L	PHB	7.3–8.4		0.39 g PHA/gVSS	Morgan-Sagastume et al., 2015

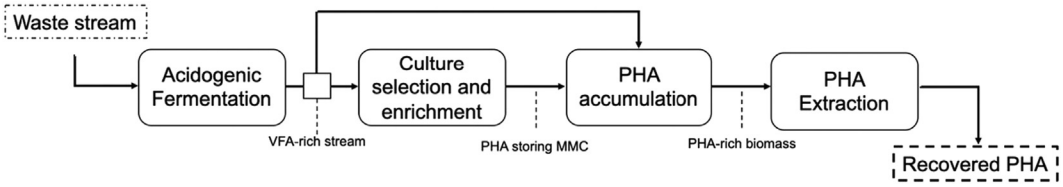

Fig. 2. The three step PHA production process using MMCs.

3.2. Mixed microbial communities

Pure cultures have been the primary focus of both laboratory and industrial scale PHA production. However, an increasing trend for deployment of mixed microbial communities (MMC) is observed in the recent decade. The possibility of consuming a wider range of carbon sources such as agricultural and industrial wastes, fewer process control parameters and lower operational and maintenance costs due to lack of sterilization, put microbial mixed cultures in a superior position in PHA production processes compared to pure cultures. A number of accumulating microorganisms such as *Azoarcus* spp., *Thauera* spp., *Paracoccus* spp., *Zoogloea and Plasticicunulans* have been identified as prominent players in MMC PHA production (Huang et al., 2018; Inoue et al., 2018; Marang et al., 2018; Wang et al., 2017). Research works have shown the PHA yields obtained from MMCs up to 89 wt% of the total suspended solids (TSS) (Johnson et al., 2009), which are comparable with pure culture production. Table 1 presents pure (both wild type and recombinant) bacteria and Table 2 summarizes some of the MMCs used for laboratory and pilot scale PHA production. While using MMCs can bring about a breakthrough in commercial PHA production due to costs reduction, the drawback is the inconsistent properties of the obtained biopolymers due to the diversity of the present microorganisms, each species producing different PHA monomers (Koller et al., 2017). Further studies on the structure of the microbial community are required in order to comprehend the dynamics and subsequent controlling of the process.

The PHA production process from MMCs typically encompasses three steps (Fig. 2):

1) Production of a feedstock enriched with readily biodegradable organic substances. The preferable feedstock is rich in VFAs which are produced from acidogenic fermentation (feedstock provision will be discussed in section 6).
2) Enrichment of the MMC with high potential PHA accumulating species
3) PHA accumulation

In mixed culture operations, a selective environmental pressure can result in obtaining a biomass with high PHA storing potential. The microbial community is exposed to alternating feast and famine regimes to enforce the domination of PHA accumulating strains. The external carbon source is provided in abundance during the feast phase while, it becomes scarce in famine phase. During the famine phase, expression of the growth associated enzymes is reduced and when the organic substances become accessible again, bacterial strains that are capable of compiling PHAs as carbon storage, are in a superior position and become dominant (Koller et al., 2017; Serafim et al., 2008; Villano et al., 2014). This step is known as culture enrichment. The culture enrichment step is very crucial due to the fact that the dominant microorganisms could affect the PHA yield and composition. Various parameters such as feedstock composition, organic loading rate (OLR) and the feast and famine ratio (F/F ratio) are of significance in the culture enrichment step (Valentino et al., 2017). Microbial population is affected by the feedstock composition. In a study by Huang et al., (2020), the genus *Paracoccus* had the highest relative abundance in the microbial community when the VFA mixture used as substrate was dominated by acetic or butyric acid. Whereas, the genus

Thauera had a higher relative abundance when the feed was enriched in propionic acid. This is due to the fact that different PHA accumulating microorganisms may have varying preferences when it comes to substrates. The results of Albuquerque et al., (2013), suggests that *Thauera* has a substrate uptake preference for propionic acid, while *Paracoccus* possess strong uptake abilities for the different VFAs present in fermented molasses except acetic acid. Furthermore, the genus *Plasticicumulans* has been observed to dominate the bacterial community when acetic acid is fed but also has high specific uptake rates for other VFAs as well (Jiang et al., 2011a; Marang et al., 2013).

On an interesting note, recent studies have shown albeit having a strict famine phase in the SBR, the enrichment of PHA producers in the MMC was not deterred by continuous supplementation of the substrate. Therefore, only a true feast phase is required (Marang et al., 2018). These results open new avenues in the scale up of MMC processes as SBR systems can be used as two step CSTR systems for PHA production.

Besides the composition of the feedstock, another important parameter during the culture enrichment step is OLR. Decreasing OLR can lead to an increase in the specific PHA production rate. This is due to the fact that alterations in OLR affects the F/F ratio, hence the microbial community. In a study by Carvalho et al., (2014), the genera *Azoarcus*, *Thaurea* and *Paracoccus* dominated an MMC fed by fermented molasses. The dominancy of *Thaurea* and *Paracoccus* was influenced by OLR.

After growing robust microorganisms with PHA storage capabilities, the next step is PHA accumulation which is achieved by exposure of the biomass to prolonged feast phase. In this step, a surplus of carbon substrate is provided while supplementation of other nutrients e.g. nitrogen or phosphorous is limited, maintaining high PHA storage until a saturation level is reached (Nielsen et al., 2017; Valentino et al., 2017; Villano et al., 2014). The objective of nutrient limitation is to shift the metabolic pathways from cell growth towards PHA production by the constriction of the TCA cycle, which is a competitive pathway (Cavaillé et al., 2013).

Carbon source, carbon to nitrogen ratio (C/N), carbon to phosphorous (C/P) ratio and pH are some of the influential parameters in PHA accumulation step (Rodriguez-Perez et al., 2018) that will be discussed in the next section.

4. Factors affecting PHA production

4.1. Carbon-nitrogen (C/N) and carbon-phosphorous (C/P) ratio

In general, low C/N ratio is beneficial for cell growth while higher C/N ratios boost PHA accumulation. The reason for acquiring higher PHA contents in N-limitation operations can be that microbial protein synthesis is inhibited and PHA is developed as the main product in the lack of nitrogen (Wen et al., 2010). The effect of C/N ratio on the biomass production stage was examined by Johnson et al., (2010). Higher PHA accumulation capability was exhibited in the microbial community when operated in carbon limited conditions (6–13C mol/N mol ratios) as opposed to nitrogen limited conditions (15–24C mol/N mol ratios). The authors concluded nitrogen limitation is favorable for PHA accumulation. However, carbon limitation is better for the enrichment step and recommended that nutrient supplementation to wastewater is necessary for the production of stable PHA storing biomass. Moreover, the influence of nitrogen concentration on PHA storage ability of activated sludge was evaluated by Ince et al., (2012). Reactors were operated under nitrogen sufficient (C/N:100/12) and nitrogen deficient (C/N: 100/2) conditions for the enrichment step. Polymer storage yields were enhanced from 0.43 to 0.61 Cmmol PHA/Cmmol of substrate under nitrogen sufficient conditions. Furthermore, in a pure culture study with archeon *Haloferax mediterranei*, Cui et al. (2017) examined a wider range of C/N ratio (ranging from 5 to 65) and the highest PHA cell contents (more than 47% CDW) were obtained at C/N ratio of 35. These results therefore point out the fact that C/N nitrogen ratio may affect different steps of the PHA production and while nitrogen limitation is vital during PHA production, there is the need for sufficient nitrogen to obtain the optimal efficiency.

C/N ratio not only alters the PHA yield but also the composition of the products. In order to better control the nitrogen levels in feast and famine phases, Silva et al. (2017) studied the impact of coupled and uncoupled nitrogen feeding on the production and composition of the PHAs. Using a combination of acetic and propionic acid as substrate, nitrogen was fed to the reactors either concurrently with the VFA substrate, or at the end of the feast phase. PHA production was two times more when carbon and nitrogen were fed separately and the composition of the acquired polymer was altered with 82% increment in the hydroxyvalerate (HV) content. Comparing three different C/N ratios of 14.3, 17.9 and 22.3C mol/N mol, the PHA production was relatively similar with C/N ratio of 14.3 and 17.9 and decreased at the higher ratio of 22.3. Meanwhile, the HV content in the obtained polymers decreased from 20% to 12% by increasing the C/N from 14.3 to 17.9 3C mol/N mol. Their results suggest the possibility of manipulating the properties of the polymers by slightly altering the C/N.

Limitation of nutrients concentrations like nitrogen or phosphorous, instigates the metabolic pathways towards PHA accumulation rather than biomass growth. Montiel-Jarillo et al. (2017) evaluated the impact of carbon/nitrogen/phosphorus ratio on PHA production using activated sludge. In batch experiments, P-limited and P-excess conditions were investigated by setting the C/N/P molar ratios at 100/8.8/1.3 and 100/8.8/90.5, respectively. Under P-limitation, 42% gPHA/gVSS of PHA was obtained while, 13% g PHA/g VSS was attained when P was fed in abundance. The results of another study indicated that phosphorous limitation reduces the 3HB production, while conserving the 3HV accumulation. It is therefore evident that P-limitation influence both PHA production and composition.

4.2. pH control

Operating the enrichment and accumulation steps at different pH values has a substantial impact on the production rate as well as the composition of the produced biopolymers. For the enrichment step, a broad pH range of 5.5–9.0 is reported to be suitable for the growth of most of non-extremophilic bacteria (Padan et al., 2005). However, in the accumulation step, the optimal pH is in the range of 7.5–8.5 (Villano et al., 2010).

In a study by Villano et al. (2010), higher polymer content in the biomass (31–34% mg PHA/mg VSS) was observed at pH 7.5 – 8.5, whereas at higher alkaline pH values, lower PHA contents were acquired (21% mg PHA/mg VSS). Furthermore, the authors concluded that pH control can be a crucial tool in controlling the 3HV content of the final polymer, regardless of the feedstock composition.

Kourmentza and Kornaros, (2016) studied the influence of initial pH on the PHA accumulation step. The pH was initially adjusted to different values of 6.4, 6.9, 7.25 and 7.5 and remained uncontrolled afterwards. Setting the initial pH at 6.9 demonstrated to be preferable among the tested conditions with PHA accumulations up to 64.5% g PHA/g CDW, while only 49% PHA accumulation was obtained when the initial pH was set at 7.5. Furthermore, Montiel-Jarillo et al. (2017) examined the influence of a wider range of pH on the accumulation step using activated sludge fed with acetate. The pH was set and controlled at different values of 4, 5.5, 6.5, 7.5, 8.5 throughout the experiments, as well as one

uncontrolled pH experiment (varying between 8.9 and 9.2). Lower PHA accumulations were acquired under acidic condition range (17–23% g PHA/g VSS). Highest PHA accumulation capacity up to 44% g PHA/g VSS was acquired in the absence of pH control. This result is promising since controlling fewer operational parameters, results in a less complicated hence a less costly process.

4.3. Feedstock composition

The feedstock composition influences the total yield of PHA production. Cui et al. (2016) evaluated the effect of carbon sources on PHA production by feeding acetate, glucose and starch as the substrate during the enrichment step of three halophilic MMCs. The reactors fed by sodium acetate and glucose resulted in the PHA content of 64.7% and 60.5% CDW, respectively. While only 27.3% CDW of PHA was obtained from the starch-enriched microbial community. The reason is because of the more complex structure of starch which cannot be directly exploited and first needs to be converted to simpler sugars. Whereas, acetate and glucose can be more easily and rapidly consumed in the metabolic pathways.

Feedstock characteristics also determines the type and composition of the produced polymers. Jiang et al. (2011b) studied the impact of substrate on PHA content and compositions. Different ratios of acetate and propionate mixture (100/0, 75/25, 50/50, 0/100 Cmol basis) were fed to the mixed microbial culture. The homopolymer PHB was observed in the experiments where acetate was the sole substrate and a copolymer with the composition of 11 Cmol% 3HB and 89 Cmol% 3HV was yielded when only propionate was fed. Their results revealed the immense dependency of the final polymer composition on the composition of the substrate. Increasing the 3HV fraction in the poly(3-hydroxybutyrate-co-3-hydroxyvalerate) polymer is of great interest. Higher 3HV contents increases the flexibility and resistance of the polymers hence, widening the applications range. *Cupriavidus necator* has the ability of generating 3HV monomers from odd carbon-number feedstocks such as propionic and valeric acid. Feeding solely propionic acid to *cupriavidus necator*, 50% mol_{3HV}/ mol $_{PHA}$ was obtained. While feeding mixture of propionic and butyric acid, only 32% mol_{3HV}/ mol $_{PHA}$ was acquired (Grousseau et al., 2014). This is a clear indication that the composition and yield of PHA can be manipulated by changing the feedstock characteristics.

As mentioned earlier, the feedstock composition can also alter the dominancy of specific genera in the enrichment step of MMC operations (section 4.2). Carvalho et al., (2018) investigated the use of alternating cheap feedstocks for PHA production. Alterations in the feedstock led to changes in the dominating phylum in the microbial community. Using sugarcane molasses, *Actinobacteria* dominated the acidogenic MMC while *Firmicutes* was dominant when cheese whey was fed. Furthermore, some less known PHA-producers genera such as *Paenibacillus* and *Lysinibacillus* were detected in the long-term operation of cheese whey feeding. The PHA storing performance was rather similar in spite of the changes in the microbial community.

5. Waste streams used as carbon source

The carbon source is of paramount importance in the PHA production process as it influences not only the yield and composition of the acquired biopolymers (Raza et al., 2018), but also constitutes approximately half of the total production costs (Schmidt et al., 2016). High production costs due to carbon substrates can be attributed to the fact that PHA accumulation is mainly an aerobic process. Therefore, a significant amount of the provided carbon is wasted by intracellular respiration i.e. formation of CO_2 and water-soluble metabolites and less than half is consumed for cell growth and PHA accumulation (Hermann-Krauss et al., 2013). Availability of affordable and sustainable carbon sources is therefore very crucial, making waste streams as perfect carbon sources for PHA production.

Several pathways have been identified for PHA synthesis. Among the three main pathways, two of them utilize sugars as the required substrate and fatty acids are the carbon source for the third route (Fig. 1). In the case of MMCs, VFA-rich feedstocks are more preferable over sugar-based substrates as they are more accessible and yield higher conversion efficiencies for PHA production (Valentino et al., 2017). Acidogenic fermentation is the common way to attain VFAs from organic materials with more complex structures comprised of lipids, proteins and carbohydrates. As discussed earlier, the composition of the obtained VFAs stipulates the composition of the PHA monomers. Moreover, a proportional correlation between the length of the VFAs and the molecular structure of the resulting PHA monomers have been reported (Rodriguez-Perez et al., 2018). Therefore, controlling the operating parameters of acidogenic fermentation plays a pivotal role. Production and composition of the VFA through acidogenic fermentation can be optimized by pH adjustments, inhibition of methanogenesis, controlling the organic loading rate (OLR) and other operating parameters i.e., hydraulic retention time and temperature (Atasoy et al., 2018).

Utilization of waste streams enriched in highly biodegradable organic compounds is considered a plausible way to diminish the production costs. Numerous substrates ranging from industrial wastewaters and byproducts and agricultural and household waste materials such as whey (Israni et al., 2020; Pescuma et al., 2015), waste oils (Borrero-de Acuña et al., 2019; Pernicova et al., 2019; Sangkharak et al., 2020), activated wastewater sludge (Cha et al., 2016; Morgan-Sagastume et al., 2015) different lignocellulosic wastes (Al-Battashi et al., 2019; Yin et al., 2019) spent coffee grounds (Obruca et al., 2014a, 2014b), glycerol (de Meneses et al., 2020; Mohamad Fauzi et al., 2019), grape pomace (Follonier et al., 2015), olive oil production wastewater (Campanari et al., 2014; Kourmentza et al., 2015), waste polypropylene (Johnston et al., 2019) have been used in laboratory and pilot-scale experiments for the production of PHAs. Below a few examples of the most commonly studied or potential substrates are mentioned.

5.1. Whey

Whey is one of the by-products of cheese making process mainly comprised of lactose, proteins and lactic acid. It is estimated that almost half of the whey is discarded in wastewater treatment facilities (Pescuma et al., 2015). Cheese way can be valorized through dark fermentation for biohydrogen production as well as PHA production through MMCs (Colombo et al., 2019). One of the perks of using cheese whey as feedstock is that no complex pretreatment is needed prior to acidogenic fermentation which is economically beneficial. In an attempt for whole whey valorization, Israni et al., (2020) obtained PHAs from *Bacillus megaterium Ti3,* and subsequently blending them with polyethylene glycol (PEG). The resulting P3HB7PEG films demonstrated notable improvements in attributes such as roughness, hydrophilicity, protein adsorption and cytocompatibility. Their promising results exhibit the potential applications of waste derived PHAs for biomedical applications.

In previous studies, Colombo et al., (2016) compared two different fermented cheese whey as substrate for activated sludge. The obtained PHA from the first cheese whey which was comprised of 58% lactic acid, 16% acetic acid and 26% butyric acid was only PHB. While, the second cheese whey containing 6% lactic acid, 58% acetic acid, 13% butyric acid, 19% propionic acid and 4% valeric

acid resulted in a PHA composition of 40% 3HV and 60% 3HB. These results signify the dependency of the proportions of the monomers in copolymers of 3HB and 3HV on the used substrate. Previous studies had also indicated that 3HV monomers can be obtained from odd carbon numbered VFAs e.g. propionic and valeric acid (Doi et al., 1988).

Many of the research with whey as carbon source use recombinant *Escherichia coli* as many traditional PHA-producing microorganisms are not capable of direct metabolization of whey, lacking β-galactosidase activity. For instance, with a recombinant strain of *E.coli* containing *Alcaligenes latus PHA* synthesizing genes, Ahn et al. (2001) acquired PHB concentrations and PHB contents of 168 g/L and 87%, respectively. The PHB concentration and productivity were substantially increased to equal levels as experiments using glucose. The results clearly indicate that whey is a better substrate for PHA production.

Besides recombinant *Escherichia coli*, archaea have been employed to convert whey to PHA. *Haloferax mediterranei*, an extremely halophilic organism has been used as microbial host. Strict sterile conditions are not needed due to the high salinity requirements for cultivation. Koller, (2015) and Pais et al., (2016) used *Haloferax mediterranei* and attained 66% and 53% of CDW, respectively. This seems to suggest whey as a plausible carbon source for a number of PHA accumulating microorganisms.

5.2. Waste oil

Waste oil is a promising carbon source as it does not require any pre-treatment step prior to use, regardless of its origin (collected from industrial applications or households). Waste rapeseed oil, waste frying palm oil, waste frying sunflower oil and corn oil have been tested for PHA production using *Cupriavidus necator* and *Pseudomonas* strains. PHA contents ranging between 35 and 68% of CDW have been reported in the literature (Chaudhry et al., 2011; Obruca et al., 2014b). Moreover, Obruca et al., (2010) conducted experiments on waste rapeseed oil and acquired up to 80% CDW of PHA by addition of propanol as a cheap 3HV precursor in the cultivation of *Cupriavidus necator* H16.

Using waste cooking oil, Sangkharak et al., (2020) produced up to 87% CDW PHA from *Bacillus thermoamylovorans*. The obtained PHAs were further used as feedstock for biofuel production (3-hydroxyalkanoate methyl ester (3HAME)). Authors suggested that 3HAME produced from microbial PHAs can be a good blending agent in order to decrease the cetane number of blended fuels for diesel engines.

Borrero-de Acuña et al., (2019) grew a recombinant *P. putida KT2440* on waste vegetable oil. By knocking out the *tctA* gene which encodes a major enzyme in carboxylic acids transport system, authors were able to obtain 1.91 g/L of mcl-PHAs in 72 hrs. The volumetric yield was approximately two times more compared to the wild type strain.

The genus *Halomonas* is deemed propitious for PHA production from sugar-based substrates. These fast growing extremophilic strains have high resistance towards microbial contamination which diminishes the need for sterile conditions and streamlines a continuous cultivation mode (Chen and Jiang, 2018b). Pernicova et al., (2019) examined the use of lipids as substrates (waste frying oil-rapeseed and sunflower oil) for PHA production using nine different *Halomonas* strains. *Halomonas hydrothermalis* was revealed to accumulate PHB up to 62% CDW. Furthermore, their results indicated a correlation between the medium salinity and the molecular weight of the polymers.

5.3. Lignocellulosic waste

Lignocellulosic materials are primarily comprised of cellulose, hemicellulose and lignin. Bagasse, wheat straw, rice straw and wheat bran are some examples of these agricultural residues. One of the advantages of lignocellulosic wastes is their low portion carbohydrate content compared to other substrates. Carbohydrates are normally consumed for glycogen accumulation in activated sludge systems rather than PHA accumulation (Jiang et al., 2009). The drawback of using lignocellulosic waste streams is that normally a pretreatment step is required to enzymatically hydrolyze the complex materials and convert them into simple sugars. A detoxification step is also followed to take out the inhibitory compounds produced during hydrolysis (Obruca et al., 2015b). These treatment steps are a burden as they add complexity to the process and increase the total production costs as well as imposing environmental issues. In attempts for improving the economic aspects, hydrothermal pretreatment of lignocellulosic biomass has been successfully implemented (Yin et al., 2019). Using hot water pretreatment is cheap, requires no chemicals and is more environmentally friendly. Poplar biomass treated with hot water was used as the substrate and PHBV concentration s up to 637 mg/L was obtained by MMCs (Yin et al., 2019).

Waste paper constitutes a significant share of the lignocellulosic biomass in the municipal solid waste. Al-Battashi et al., (2019) exploited waste paper hydrolysate as the carbon source for *Burkholderia sacchari* cultivation and reported PHB accumulation of 44.2% CDW. In a study by, Annamalai and Sivakumar (2016), a metabolically engineered *Ralstonia eutropha NCIMB 11,599* was grown on an alkaline pretreated wheat bran hydrolysate. PHB accumulation was 62.5% of CDW. Moreover, in fed batch cultivation of *C. necator DSM 545*, Li and Wilkins, (2020) obtained 4.5 g/L PHB, which is the highest production reported so far using lignin as the substrate.

5.4. Spent coffee grounds (SCG)

The oil content of coffee grounds is estimated to be between 7 and 15% (Karmee, 2018). While, the remaining portion is mostly lignocellulosic materials that are exploited for energy production (through combustion) or they can be used as a raw material for PHAs (Nielsen et al., 2017) owing to their high carbon density (Atabani et al., 2019). Obruca et al. (2014a,b,c) evaluated the suitability of using oil extracted from SCG comparing with waste frying rapeseed oils, waste frying palm oil and waste frying sunflower oil. The obtained PHB yields and PHB contents from *Cupriavidus necator* H16 were 49.4 g/l and 89.1% CDW, respectively. These PHB yields were higher in comparison to experiments with other waste oils due to high contents of free fatty acids in spent coffee grounds.

Moreover, SCG hydrolysates which are considered as lignocellulosic wastes have been used as the cheap carbon source for pure culture PHA biosynthesis by *Burkholderia cepacia* and *Bacillus megaterium* (Obruca et al., 2014a; Stanislav Obruca et al., 2015a, b). Kovalcik et al., (2018) examined the PHA production by *Halomonas halophila from* SCGs hydrolysates. In their initial experiments, the microbial growth was utterly inhibited using different hydrolysates (non-modified, defatted and defatted with removed phenolics). Hence, the hydrolysates were detoxified with sorbents on styrene–divinylbenzene based resins, as a pre-treatment step. PHB content of 27% (wt/wt) was obtained. The acquired PHA yields are still low and the necessity of a detoxification treatment for the bacterial growth does not make this bacterium a very promising PHA producer from SCG.

5.5. Glycerol, by-product of biodiesel production

The major side product of the biodiesel generation industry is crude glycerol. Less carbon atoms are present in glycerol in comparison to carbohydrates. Hence, they have become an appealing feedstock for PHA production. Mohamad Fauzi et al., (2019) fed crude glycerol to activated sludge under different organic loading rates. The authors acquired PHAs up to 80% CDW containing 3HB and 3HV monomers at a HB:HV molar ratio of 60:40. It was indicated that by increasing the organic loading rate from 360 mgC/(L.d) to 1000 mgC/(L.d), the biomass concentration was notably incremented from 0.7 g/L to 2 g/L at the enrichment step. Furthermore, glycerol has been used for metabolically engineered species. Fukui et al., (2014) introduced the aquaglyceroporin (glpF) and glycerol kinase (glpK) from *E.coli* to *Ralstonia eutropha H16* and were able to accumulate PHA content up to 68% CDW. Moreover, in a recent study by de Meneses et al.,(2020) glycerol was the sole carbon source in simultaneous microbial synthesis of mcl-PHAs, high molecular weight extracellular polysaccharides (EPS) and phenazines (which can act as antibiotics and antitumor agents) by *Pseudomonas chlororaphis*. The PHA and EPS production were 2.23 g/L and 6.10 g/L, respectively. Obtaining only 19% CDW of PHAs, further optimization of the operational conditions is required. Nevertheless, the results are promising as the feasibility of simultaneous conversion of a single substrate into various high value-added products was illustrated.

5.6. CO_2 as carbon source

Utilization of CO_2 is another possibility for PHA production. Among the broad diversity of PHA producing bacteria, cyanobacteria are the sole photosynthetic prokaryotes that are capable of synthesizing PHB (Carr, 1966). Different studies have indicated that at least 20 species of cyanobacteria produce PHB (Bhati et al., 2010; Stal, 1992). However, the yield and accumulation of PHB in these bacteria are quite low. *Nostoc muscorum* is the cyanobacteria-specie with the highest PHB production, 8.5 (%w/w) after 21 days of cultivation (Bhati et al., 2010). Genetic modification of these bacteria is highly required to increase PHB production from CO_2.

Synthetic consortia are another approach to produce PHAs from CO_2. Weiss T. L. et al. (2017) reported a consortium of an engineered cyanobacterium *Synechococcus elongatus* PCC 7942, able to export up to 85% of photosynthetically-fixed carbon as sucrose, with *Halomonas boliviensis*, a wild-type PHA producer able to convert sucrose in PHB (Weiss et al., 2017).

Furthermore, it has been suggested that some archaeal genera such as *Metallosphaera*, *Sulfolobus*, *Archaeoglobus* and *Cenarchaeum*, have the ability to assimilate CO_2 through their carbon fixation cycles. Many of the archaea are extremophile, making them a viable candidate for utilization in concurrent reduction of gaseous compounds from industrial effluents and production of bioproducts such as PHAs (Venkata Mohan et al., 2016).

6. PHA extraction

PHA downstream processing constitutes 30% of the total production costs (Sun et al., 2007) and is of paramount importance as it stipulates the quality of the polymers and consequently their end-use and market value.

For the selection of a suitable recovery technique different parameters such as composition and type of the biopolymers, PHAs producer, end-use and product purity stipulations, expenses and environmental considerations must be contemplated. For instance regarding the PHA producer, the cell wall of recombinant species are thinner and more fragile compared to wild type PHA accumulators (Mohammadi et al., 2012) or MMCs are known to have a higher resistance to cell hydrolysis than pure cultures (Samorì et al., 2015). In general, recovery methods can be categorized in two groups: (Jacquel et al., 2008)

1) Digestion methods where the biomass is dissolved in order to separate the PHA granules

2) Direct extraction of PHA from biomass using proper solvents

The choice between dissolving the PHA or the non-PHA cell mass (NPCM) is influenced by the percentage of the accumulated polymers inside the microbial cells (Koller et al., 2013).

Digestion can be carried out through chemical or enzymatic treatment (Raza et al., 2018). In digestion methods, NPCM is dissolved, the cell wall is disrupted while the intracellularly aggravated PHA granules are preserved unharmed (Villano et al., 2014; Kosseva and Rusbandi, 2018). The PHAs are recovered and the NPCM can be further used as "green fertilizer" in agricultural sector or be fed to biogas plants for anaerobic digestion (Koller et al., 2013).

Oxidating agents such as sodium hydroxide and sodium hypochlorite (Gobi and Vadivelu, 2015) or acids such as sulfuric acid (López-Abelairas et al., 2015) have been used for chemical digestion. The concentration of the utilized chemical plays a substantial role as it can not only dissolve the NCPM but also, degrade the PHAs and culminate in low polymer recovery and decreased purity, molecular weight and mechanical strength of the polymers (Kunasundari and Sudesh, 2011; Kosseva and Rusbandi, 2018). Furthermore, the aforementioned chemicals are toxic and impose negative environmental burdens.

Solvent extraction is the most prevalent method for PHA extraction (Raza et al., 2018), in which the PHAs are directly extracted from the biomass. Normally, it is comprised of immersion of the PHA-containing biomass into a proper solvent or mixture of solvents in order to dissolve the granules, succeeded by addition of a precipitating agent to retrieve the polymers in the crystal form (Kosseva and Rusbandi, 2018). Chlorinated solvents for instance chloroform and dichloromethane, are typically utilized in the dissolution step. When extracting mcl-PHAs, acetone is the preferable solvent. For the precipitation step, methanol and ethanol are used. Solvent extraction is usually used when high purity of the polymers is desired (Jacquel et al., 2008). However, some of these solvents are not only unsustainable but also, raise serious health and environmental concerns. Due to the toxicity and detrimental effects of the halogen containing solvents on environment, researchers are looking for alternative candidates. Utilization of anisole, cyclohexanone, and phenetole as more environmentally friendly alternatives has been suggested (Rosengart et al., 2015). Samorì et al., (2015) investigated a novel extraction technique, utilizing dimethylcarbonate (DMC) as a green substitute to conventional chlorinated solvents to extract PHA from a microbial mixed culture. Polymer recoveries similar to utilization of dichloromethane were obtained without any reduction in purity or molecular weight.

In the case of MMCs, utilization of chlorinated extracting agents is less effective compared to pure culture cultivations. This can be attributed to more complex and resistant extracellular biomass matrix that encompasses the PHAs (Patel et al., 2009). Novel extraction techniques have been proposed by using switchable anionic surfactants (SAS) as a sustainable substitute for surfactants which is more economical and less solvent intensive (Mannina et al., 2019).

Recently, researchers are experimenting on novel biological approaches such as PHA recovery by insects. Zainab-L and Sudesh, (2019) fed *C. necator H16* cells to mealworms and recovered the PHA granules (82% PHA content) from their feces. In previous studies, Murugan et al., (2016) were able to retrieve almost 100% pure PHA granules after performing further purification steps

on the fecal pellets. Their results demonstrated that the molecular weight and dispersion of the recovered PHAs were similar to granules extracted using chloroform. In another study, Kunasundari et al., (2017) used laboratory rats to recover PHB. Lyophilized cells of *C. necator H16* were fed to laboratory rats and the excreted fecal pellets were comprised of 82–97 wt% of PHB. The rest of the impurities were cleaned without using any strong chemicals as solvent. Moreover, there has been a study whereby a bacteriophage was used as a bioextractant. Phage-mediated lysis successfully extracted approximately two thirds of the PHB contained within *P. oleovorans* cells grown on glycerol (Hand et al., 2016). These biological PHA extraction approaches seem innovative, however, the ethical issues and their applicability in large scale operations should also be looked at holistically.

7. Current challenges and future perspectives

Microbiologically produced PHAs are a viable candidate to substitute conventional petroleum-derived plastics because of their similar properties as well as astonishing attributes such as biodegradability and biocompatibility. Promising findings on PHAs production have been reported in the recent years. Yet, the big question is what are the main hurdles for their large-scale production? At an industrial scale, the robustness of any biochemical process is assessed based on yield, production rate, product titer and efficiency of downstream processing in combination with costs considerations (Noorman and Heijnen, 2017). In the case of PHAs, the production costs are considerably higher in comparison to traditional polymers such as polypropylene. A number of challenges should be addressed to decline the production costs and make PHAs commercially competitive to traditional plastics:

7.1. Sustainable and cheap carbon sources

As almost 50% of the production costs are ascribed to raw material provision (Choi and Lee, 1997), utilization of cheaper substrates is crucial for commercialization of PHA production. Availability of the substrate in sufficient quantities throughout the year with similar qualities is one of the prerequisites for a proper substrate, particularly for large scale operations. Also, the logistics for collection and transportation of the feedstock can also impose some costs. One proposed solution to lower the costs is the utilization of the industrial waste streams (such as food processing, pulp and paper, etc.) by integrating PHA production in the existing industrial facilities and transforming them into multiple-producing biorefineries. Doing so, can provide continuous substrate and microbial community for PHA production and eliminate transportation costs. Moreover, this process integration closes the loop of material consumption cycles through reuse and recycling of the waste (Nguyen et al., 2018). PHA production can be integrated in the existing wastewater treatment facilities, agricultural or food manufacturing facilities and biodiesel factories. This resource recovery approach also complies well with the United Nation's sustainable development goals and zero waste policies. As an example, Tomei et al., (2016) examined the potential impacts of turning a municipal wastewater treatment plant as a supplier of PHA-rich biomass from an LCA perspective. Four different configurations estimated to have up to 40% and 90% less global warming potential and terrestrial eutrophication potential, respectively. The results of the environmental impact assessments, encourage the concept of converting a municipal WWTP as sustainable supplier of value-added renewable materials other than merely biogas production.

One successful example of PHA integration in WWTPs in pilot scale, is the PHARIO project. Thermally stable PHAs were produced from surplus activated sludge fed with VFAs from primary sludge and industrial liquors with yields up to 0.41 gPHA/gVSS. Operating throughout the four seasons, it was substantiated that surplus activated sludge can be a trustable source for large-scale PHA productions with consistent product quality (Werker et al., 2018). Furthermore, from the LCA point of view it was demonstrated that the environmental impact of the obtained biopolymers within the PHARIO project is 70% less than pure culture PHAs (Bengsston et al., 2017). Another example is the integration of concurrent phosphorus recovery, nitrification and PHA production on demo scale (7.8 m^3) in municipal WWTP in Barcelona (Larriba et al., 2020). However, the PHA accumulation was not sufficiently high for an economically-sensible extraction in this new system. It was concluded that PHA content can be enhanced by manipulation of sludge retention time and VFA feeding and for now, the obtained sludge containing PHA can be fed to anaerobic digestors, improving their biogas generation.

In lab scale, providing a substrate with the same composition can be easily achieved. However, one of the constraints of upscaling PHA production is maintaining the productivity of the process as well as the product quality, since ample amount of carbon sources is required. One of the concerns about utilization of industrial byproducts as the carbon source, is their fluctuating composition with seasonal changes which can extremely influence the PHA production process. Resiliency of the PHA production process by feeding alternating seasonal industrial byproducts was validated in a recent study by Carvalho et al., (2018). Initially, using fermented molasses and later switching to cheese whey as substrate, after reaching pseudo steady state, similar PHA storing performance was observed. This indicated the reliability of robust MMC operations, albeit provision of varying feedstocks.

7.2. Enhancing the process efficiency

Low substrate conversion, inconsistent structure and properties in each batch and difficulties associated with downstream processing are some of the other bottlenecks of PHA commercialization. A robust bioprocess design in combination with deployment of tools such as metabolic engineering and synthetic biology are deemed as promising approaches to obviate these barriers.

Metabolic engineering of pathways is one of the solutions to enhance the low conversion of substrates. For instance, eliminating the *fadA* and *fadB* enzymes in the beta oxidation pathway of fatty acids, weakens the competing pathways with PHA synthesis therefore, the metabolic flux towards PHA synthesis is augmented (Chen and Jiang, 2018a; Chen and Jiang, 2017). Elimination of the side products in the pathways is also of interest to increase the PHA synthesis efficiency. Furthermore, manipulation of cell division pattern is another appealing technique to enhance the PHA production process that can be achieved through alteration in cell growth system and converting the binary fission to multiple fission system by gene repression (Chen and Jiang, 2017). Utilization of the CRISPR/Cas 9 technology is opening new windows in manipulating the genes associated to PHA synthesis and regulating the PHA structure and molecular weights (Chen and Jiang, 2018a). Lv et al. (2015) attempted fine-tuning of prokaryotic gene expression by using CRISPRi. Regulating the pathway flux towards PHA synthesis and controlling the PHA composition in *Escherichia coli,* their results indicated the feasibility of simultaneous manipulation of multiple genes in *E. coli.*

Inconsistent polymer structures, unreliable monomer ratios and obtaining PHAs with different molecular weights from batch to batch is the consequence of oscillations in the PHA synthase (PhaC) activity. Controlling the transcription of the PhaC gene culminates in regulation of the PhaC enzyme activity (Chen and Jiang, 2017).

In the case of MMCs, research must be conducted in the application of bioaugmentation strategies to enhance the production yield. Bioaugmentation is the introduction of a particular consortia or microbial strains in the system to intensify an existing population and promote a desired activity (Maier and Gentry, 2014). Bioaugmentation of the microbial community has been successfully implemented and improved the efficiency of both aerobic and anaerobic processes such as biological phosphorous removal (Yadav et al., 2020) and biogas production (Jiang et al., 2020; Tian et al., 2019). Regarding PHA synthesis, through bioaugmentation of *Plasticicumulans acidivorans* dominated biomass, accumulation of PHB contents up to 85% from mixed culture operations have been achieved (Marang et al., 2018), which is almost as high as the yield from potent pure culture microorganisms. By addition of vigorous PHA accumulating strains into mixed cultures, higher conversion of the raw products to biopolymers can be obtained, making commercial MMC PHA production closer to reality.

One of the other barriers of scaling up the biochemical processes, is the aeration requirements. Recently, the use of phototrophic mixed cultures (PMCs) for PHA synthesis is also drawing attention. Aeration is not required for the growth of phototrophic organisms, as they form ATP from the energy absorbed from sunlight instead of oxygen. Obviating the associated costs of aeration systems, PMCs present new opportunities for potential large scale applications. Fradinho et al., (2019) illustrated for the first time that PMCs have the ability to incorporate cheese whey into PHAs with 12% HV content. Their results are promising for utilization of real waste streams for PHA production from PMCs. The same research group have also proposed a PMC consortium comprised of bacteria and algae as opposed to MMC operations, which can lead to cost reduction of PHA productions (Fradinho et al., 2013).

7.3. Extraction

Almost one third of the total PHA production costs are ascribed to the extraction step (Sun et al., 2007). The use of cheaper and sustainable methods for extraction of the accumulated PHAs will considerably reduce the PHA total costs. Chemicals are extensively consumed in the PHA extraction processes as digestion, solvent extraction and floatation methods all use chemicals. Additional costs of solvent disposal or regeneration is a drawback. The cost of PHA production has been estimated to be 2–3 times more expensive compared to petrochemical plastics by using chlorinated solvents or surfactants (Yu and Chen, 2006). Furthermore, generation of hazardous wastes during extraction is contradictory to the environmentally friendly nature of the PHA production. The upcoming researches should focus on utilization of less expensive and more environmentally friendly chemicals and solvents.

Moreover, increasing the recovery efficiency is another extraction aspect that needs to be addressed. To ease the downstream processing, efforts should be made in morphology engineering to alter the size and shape of the PHA granules. Another suggestion is deletion of some of the genes associated to cell wall synthesis (Wang et al., 2014b) or promotion of secretion pathways for extracellular deposition of PHAs as opposed to intracellular accumulation. Rahman et al., (2013) investigated the feasibility of secretion of PHB by targeting the binding proteins to the outer layer of the granules (Phasin and PhaP1). Establishment of mechanisms for extracellular accumulation of PHAs presents new research opportunities. Extracellular accumulation not only streamlines the recovery process but also increases the accumulation capacity as it won't be constrained to the small cell volume.

8. Conclusions

Currently, commercialization of bioplastics is at a pivotal point. Recent stringent regulations on the ban or minimization of the conventional petroleum derived plastics, the projected decrease in fossil fuel resources and public awareness are propelling the pursuit of biodegradable and sustainable plastics. Therefore, microbial production of polyhydroxyalkanoates is becoming more prevalent due to its benefits. In order to ensure sustainable PHA production, the main challenges to overcome are the costs of the carbon source as well as enhancing the production and extraction efficiency. Utilization of different waste streams as the required carbon source will not only diminish the production costs, but also closes the loop of material consumption cycles which is one of the pillars of the realization of circular economy. Application of waste by-products and mixed microbial cultures in conjunction with metabolic engineering tools are provisioned as the way forward in PHA production processes. The bottleneck of the mixed microbial cultures, is the erratic properties of the acquired biopolymers. Currently, most of research works are concentrated on increasing the yield of the process. However, to convince the market to accept PHAs from MMCs, the focus of the research should shift towards gaining the same quality with consistent composition of the biopolymer products and not only high production yields. Bioaugmentation strategy as well as effective extraction methods can be the vehicle to push MMC-based PHA production forward.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The study was part of the CarbonNextGen project (Project No: 46119-1) financed by the Swedish Energy Agency in the scope of RE:Source Strategic Inovation Programme.

References

Agnew, D.E., Pfleger, B.F., 2013. Synthetic biology strategies for synthesizing polyhydroxyalkanoates from unrelated carbon sources. Chem. Eng. Sci. https://doi.org/10.1016/j.ces.2012.12.023.

Ahn, W.S., Park, S.J., Lee, S.Y., 2001. Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant Escherichia coli. Biotechnol. Lett. 23, 235–240. https://doi.org/10.1023/A:1005633418161.

Al-Battashi, H., Annamalai, N., Al-Kindi, S., Nair, A.S., Al-Bahry, S., Verma, J.P., Sivakumar, N., 2019. Production of bioplastic (poly-3-hydroxybutyrate) using waste paper as a feedstock: Optimization of enzymatic hydrolysis and fermentation employing Burkholderia sacchari. J. Clean. Prod. 214, 236–247. https://doi.org/10.1016/j.jclepro.2018.12.239.

Albuquerque, M.G.E., Carvalho, G., Kragelund, C., Silva, A.F., Barreto Crespo, M.T., Reis, M.A.M., Nielsen, P.H., 2013. Link between microbial composition and carbon substrate-uptake preferences in a PHA-storing community. ISME J. https://doi.org/10.1038/ismej.2012.74.

Albuquerque, P.B.S., Malafaia, C.B., 2018. Perspectives on the production, structural characteristics and potential applications of bioplastics derived from polyhydroxyalkanoates. Int. J. Biol. Macromol. 107, 615–625. https://doi.org/10.1016/j.ijbiomac.2017.09.026.

Anfelt, J., Kaczmarzyk, D., Shabestary, K., Renberg, B., Rockberg, J., Nielsen, J., Uhlén, M., Hudson, E.P., 2015. Genetic and nutrient modulation of acetyl-CoA levels in Synechocystis for n-butanol production. Microb. Cell Fact. https://doi.org/10.1186/s12934-015-0355-9.

Anjum, A., Zuber, M., Zia, K.M., Noreen, A., Anjum, M.N., Tabasum, S., 2016. Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2016.04.069.

Annamalai, N., Sivakumar, N., 2016. Production of polyhydroxybutyrate from wheat bran hydrolysate using Ralstonia eutropha through microbial fermentation. J. Biotechnol. 237, 13–17. https://doi.org/10.1016/j.jbiotec.2016.09.001.

Atabani, A.E., Al-Muhtaseb, A.H., Kumar, G., Saratale, G.D., Aslam, M., Khan, H.A., Said, Z., Mahmoud, E., 2019. Valorization of spent coffee grounds into biofuels and value-added products: Pathway towards integrated bio-refinery. Fuel. https://doi.org/10.1016/j.fuel.2019.115640.

Atasoy, M., Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2018. Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2018.07.042.

Bengsston, S., Werker, A., Visser, C., Korving, L., 2017. PHARIO Stepping stone to a sustainable vaue chain for PHA bioplastic using municipal activated sludge. STOWA Rep.

Bhalerao, A., Banerjee, R., Nogueira, R., 2020. Continuous cultivation strategy for yeast industrial wastewater-based polyhydroxyalkanoate production. J. Biosci. Bioeng. https://doi.org/10.1016/j.jbiosc.2019.11.006.

Bhati, R., Samantaray, S., Sharma, L., Mallick, N., 2010. Poly-β-hydroxybutyrate accumulation in cyanobacteria under photoautotrophy. Biotechnol. J. https://doi.org/10.1002/biot.201000252.

Borrero-de Acuña, J.M., Aravena-Carrasco, C., Gutierrez-Urrutia, I., Duchens, D., Poblete-Castro, I., 2019. Enhanced synthesis of medium-chain-length poly(3-hydroxyalkanoates) by inactivating the tricarboxylate transport system of Pseudomonas putida KT2440 and process development using waste vegetable oil. Process Biochem. https://doi.org/10.1016/j.procbio.2018.10.012.

Burniol-Figols, A., Varrone, C., Daugaard, A.E., Le, S.B., Skiadas, I.V., Gavala, H.N., 2018. Polyhydroxyalkanoates (PHA) production from fermented crude glycerol: Study on the conversion of 1,3-propanediol to PHA in mixed microbial consortia. Water Res. https://doi.org/10.1016/j.watres.2017.10.046.

Cai, L., Yuan, M.Q., Liu, F., Jian, J., Chen, G.Q., 2009. Enhanced production of medium-chain-length polyhydroxyalkanoates (PHA) by PHA depolymerase knockout mutant of Pseudomonas putida KT2442. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2008.11.020.

Campanari, S., E Silva, F.A., Bertin, L., Villano, M., Majone, M., 2014. Effect of the organic loading rate on the production of polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2014.06.006

Carr, N.G., 1966. The occurrence of poly-β-hydroxybutyrate in the blue-green alga. BBA - Biophys. Incl. Photosynth. https://doi.org/10.1016/0926-6585(66)90353-0.

Carvalho, G., Oehmen, A., Albuquerque, M.G.E., Reis, M.A.M., 2014. The relationship between mixed microbial culture composition and PHA production performance from fermented molasses. N. Biotechnol. https://doi.org/10.1016/j.nbt.2013.08.010.

Carvalho, G., Pedras, I., Karst, S.M., Oliveira, C.S.S., Duque, A.F., Nielsen, P.H., Reis, M.A.M., 2018. Functional redundancy ensures performance robustness in 3-stage PHA-producing mixed cultures under variable feed operation. N. Biotechnol. https://doi.org/10.1016/j.nbt.2017.08.007.

Cavaillé, L., Grousseau, E., Pocquet, M., Lepeuple, A.-S., Uribelarrea, J.-L., Hernandez-Raquet, G., Paul, E., 2013. Polyhydroxybutyrate production by direct use of waste activated sludge in phosphorus-limited fed-batch culture. Bioresour. Technol. 149, 301–309. https://doi.org/10.1016/j.biortech.2013.09.044.

Cha, S.H., Son, J.H., Jamal, Y., Zafar, M., Park, H.S., 2016. Characterization of polyhydroxyalkanoates extracted from wastewater sludge under different environmental conditions. Biochem. Eng. J. https://doi.org/10.1016/j.bej.2015.12.021.

Chaudhry, W.N., Jamil, N., Ali, I., Ayaz, M.H., Hasnain, S., 2011. Screening for polyhydroxyalkanoate (PHA)-producing bacterial strains and comparison of PHA production from various inexpensive carbon sources. Ann. Microbiol. https://doi.org/10.1007/s13213-010-0181-6.

Chen, G.-Q., 2009. A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. Chem. Soc. Rev. 38, 2434–2446. https://doi.org/10.1039/B812677C.

Chen, G.-Q., Jiang, X.-R., 2018a. Engineering microorganisms for improving polyhydroxyalkanoate biosynthesis. Curr. Opin. Biotechnol. 53, 20–25. https://doi.org/10.1016/j.copbio.2017.10.008.

Chen, G.Q., Jiang, X.R., 2018b. Next generation industrial biotechnology based on extremophilic bacteria. Curr. Opin. Biotechnol. https://doi.org/10.1016/j.copbio.2017.11.016.

Chen, G.-Q., Jiang, X.-R., 2017. Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis. Synth. Syst. Biotechnol. 2, 192–197. https://doi.org/10.1016/j.synbio.2017.09.001.

Chen, G.Q., 2010. Plastics from Bacteria. Microbiology Monographs. https://doi.org/10.1007/978-3-642-03287_5_3.

Choi, J. Il, Lee, S.Y., 1997. Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. Bioprocess Eng. https://doi.org/10.1007/s004490050394.

Colombo, B., Pepè Sciarria, T., Reis, M., Scaglia, B., Adani, F., 2016. Polyhydroxyalkanoates (PHAs) production from fermented cheese whey by using a mixed microbial culture. Bioresour. Technol. 218, 692–699. https://doi.org/10.1016/j.biortech.2016.07.024.

Colombo, B., Villegas Calvo, M., Pepè Sciarria, T., Scaglia, B., Savio Kizito, S., D'Imporzano, G., Adani, F., 2019. Biohydrogen and polyhydroxyalkanoates (PHA) as products of a two-steps bioprocess from deproteinized dairy wastes. Waste Manag. https://doi.org/10.1016/j.wasman.2019.05.052.

Cui, Y.-W., Shi, Y.-P., Gong, X.-Y., 2017. Effects of C/N in the substrate on the simultaneous production of polyhydroxyalkanoates and extracellular polymeric substances by Haloferax mediterranei via kinetic model analysis. RSC Adv. 7, 18953–18961.

Cui, Y.W., Zhang, H.Y., Lu, P.F., Peng, Y.Z., 2016. Effects of carbon sources on the enrichment of halophilic polyhydroxyalkanoate-storing mixed microbial culture in an aerobic dynamic feeding process. Sci. Rep. https://doi.org/10.1038/srep30766.

de Meneses, L., Pereira, J.R., Sevrin, C., Grandfils, C., Paiva, A., Reis, M.A.M., Freitas, F., 2020. Pseudomonas chlororaphis as a multiproduct platform: Conversion of glycerol into high-value biopolymers and phenazines. N. Biotechnol. https://doi.org/10.1016/j.nbt.2019.10.002.

de Paula, F.C., Kakazu, S., de Paula, C.B.C., Gomez, J.G.C., Contiero, J., 2017. Polyhydroxyalkanoate production from crude glycerol by newly isolated Pandoraea sp. Polyhydroxyalkanoate production from crude glycerol. J. King Saud Univ. – Sci. https://doi.org/10.1016/j.jksus.2016.07.002.

Dietrich, K., Dumont, M.-J., Del Rio, L.F., Orsat, V., 2017. Producing PHAs in the bioeconomy — Towards a sustainable bioplastic. Sustain. Prod. Consum. 9, 58–70. https://doi.org/10.1016/j.spc.2016.09.001.

Doi, Y., Tamaki, A., Kunioka, M., Soga, K., 1988. Production of copolyesters of 3-hydroxybutyrate and 3-hydroxyvalerate by Alcaligenes eutrophus from butyric and pentanoic acids. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/BF00268190.

Domingos, J.M.B., Puccio, S., Martinez, G.A., Amaral, N., Reis, M.A.M., Bandini, S., Fava, F., Bertin, L., 2018. Cheese whey integrated valorisation: Production, concentration and exploitation of carboxylic acids for the production of polyhydroxyalkanoates by a fed-batch culture. Chem. Eng. J. https://doi.org/10.1016/j.cej.2017.11.024.

Emadian, S.M., Onay, T.T., Demirel, B., 2017. Biodegradation of bioplastics in natural environments. Waste Manag. https://doi.org/10.1016/j.wasman.2016.10.006.

EUPPD, 2019. Directive 2019/904 of the European Parliament and of the council on the reduction of the impact of certain plastic products on the environment. Off. J. Eur. Union.

European Bioplastics, 2018. Bioplastics market data 2018: Global production capacities of bioplastics 2018-2023. European Bioplastics, Berlin.

Faccin, D.J.L., Rech, R., Secchi, A.R., Cardozo, N.S.M., Ayub, M.A.Z., 2013. Influence of oxygen transfer rate on the accumulation of poly(3-hydroxybutyrate) by Bacillus megaterium. Process Biochem. https://doi.org/10.1016/j.procbio.2013.02.004.

Follonier, S., Riesen, R., Zinn, M., 2015. Pilot-scale production of functionalized mcl-PHA from grape pomace supplemented with fatty acids. Chem. Biochem. Eng. Q. https://doi.org/10.15255/CABEQ.2014.2251.

Fontaine, P., Mosrati, R., Corroler, D., 2017. Medium chain length polyhydroxyalkanoates biosynthesis in Pseudomonas putida mt-2 is enhanced by co-metabolism of glycerol/octanoate or fatty acids mixtures. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2017.01.115.

Fradinho, J.C., Domingos, J.M.B., Carvalho, G., Oehmen, A., Reis, M.A.M., 2013. Polyhydroxyalkanoates production by a mixed photosynthetic consortium of bacteria and algae. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2013.01.050.

Fradinho, J.C., Oehmen, A., Reis, M.A.M., 2019. Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2018.12.270.

Fukui, T., Mukoyama, M., Orita, I., Nakamura, S., 2014. Enhancement of glycerol utilization ability of Ralstonia eutropha H16 for production of polyhydroxyalkanoates. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-014-5831-3.

Gobi, K., Vadivelu, V.M., 2015. Polyhydroxyalkanoate recovery and effect of in situ extracellular polymeric substances removal from aerobic granules. Bioresour. Technol. 189, 169–176.

González-Villanueva, M., Galaiya, H., Staniland, P., Staniland, J., Savill, I., Wong, T.S., Tee, K.L., 2019. Adaptive Laboratory Evolution of Cupriavidus necator H16 for Carbon Co-Utilization with Glycerol. Int. J. Mol. Sci. 20, 5737. https://doi.org/10.3390/ijms20225737.

Groh, K.J., Backhaus, T., Carney-Almroth, B., Geueke, B., Inostroza, P.A., Lennquist, A., Leslie, H.A., Maffini, M., Slunge, D., Trasande, L., Warhurst, A.M., Muncke, J., 2019. Overview of known plastic packaging-associated chemicals and their hazards. Sci. Total Environ. 651, 3253–3268. https://doi.org/10.1016/j.scitotenv.2018.10.015.

Grousseau, E., Blanchet, E., Déléris, S., Albuquerque, M.G.E., Paul, E., Uribelarrea, J.L., 2014. Phosphorus limitation strategy to increase propionic acid flux towards 3-hydroxyvaleric acid monomers in Cupriavidus necator. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2013.11.072.

Guzmán, H., Van-Thuoc, D., Martín, J., Hatti-Kaul, R., Quillaguamán, J., 2009. A process for the production of ectoine and poly(3-hydroxybutyrate) by Halomonas boliviensis. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-009-2036-2.

Han, J., Hou, J., Liu, H., Cai, S., Feng, B., Zhou, J., Xiang, H., 2010. Wide distribution among halophilic archaea of a novel polyhydroxyalkanoate synthase subtype with homology to bacterial type III synthases. Appl. Environ. Microbiol. 76, 7811–7819. https://doi.org/10.1128/AEM.01117-10.

Hand, S., Gill, J., Chu, K.-H., 2016. Phage-based extraction of polyhydroxybutyrate (PHB) produced from synthetic crude glycerol. Sci. Total Environ. 557–558, 317–321. https://doi.org/10.1016/j.scitotenv.2016.03.089.

Hänggi, U.J., 1990. Pilot scale production of PHB with Alcaligenes latus, in: Novel Biodegradable Microbial Polymers. https://doi.org/10.1007/978-94-009-2129-0_6

Hassan, M.A., Yee, L.-N., Yee, P.L., Ariffin, H., Raha, A.R., Shirai, Y., Sudesh, K., 2013. Sustainable production of polyhydroxyalkanoates from renewable oil-palm

biomass. Biomass and Bioenergy 50, 1–9. https://doi.org/10.1016/j.biombioe.2012.10.014.
Hermann-Krauss, C., Koller, M., Muhr, A., Fasl, H., Stelzer, F., Braunegg, G., 2013. Archaeal production of polyhydroxyalkanoate (PHA) Co- and terpolyesters from biodiesel industry-derived by-products. Archaea. https://doi.org/10.1155/2013/129268.
Huang, L., Chen, Z., Wen, Q., Ji, Y., Wu, Z., Lee, D.J., 2020. Toward flexible regulation of polyhydroxyalkanoate composition based on substrate feeding strategy: Insights into microbial community and metabolic features. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.122369.
Huang, L., Chen, Z., Wen, Q., Zhao, L., Lee, D.J., Yang, L., Wang, Y., 2018. Insights into Feast-Famine polyhydroxyalkanoate (PHA)-producer selection: Microbial community succession, relationships with system function and underlying driving forces. Water Res. https://doi.org/10.1016/j.watres.2017.12.033.
Impallomeni, G., Ballistreri, A., Carnemolla, G.M., Rizzo, M.G., Nicolò, M.S., Guglielmino, S.P.P., 2018. Biosynthesis and structural characterization of polyhydroxyalkanoates produced by Pseudomonas aeruginosa ATCC 27853 from long odd-chain fatty acids. Int. J. Biol. Macromol. 108, 608–614. https://doi.org/10.1016/j.ijbiomac.2017.12.037.
Ince, O., Basak, B., Ince, B.K., Cetecioglu, Z., Celikkol, S., Kolukirik, M., 2012. Effect of nitrogen deficiency during SBR operation on PHA storage and microbial diversity. Environ. Technol. (United Kingdom) 33, 1827–1837. https://doi.org/10.1080/09593330.2011.634441.
Inoue, D., Suzuki, Y., Sawada, K., Sei, K., 2018. Polyhydroxyalkanoate accumulation ability and associated microbial community in activated sludge-derived acetate-fed microbial cultures enriched under different temperature and pH conditions. J. Biosci. Bioeng. 125, 339–345. https://doi.org/10.1016/j.jbiosc.2017.09.008.
Israni, N., Venkatachalam, P., Gajaraj, B., Varalakshmi, K.N., Shivakumar, S., 2020. Whey valorization for sustainable polyhydroxyalkanoate production by Bacillus megaterium: Production, characterization and in vitro biocompatibility evaluation. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2019.109884.
Jacquel, N., Lo, C.-W., Wei, Y.-H., Wu, H.-S., Wang, S.S., 2008. Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochem. Eng. J. 39, 15–27. https://doi.org/10.1016/j.bej.2007.11.029.
Jarmander, J., Belotserkovsky, J., Sjöberg, G., Guevara-Martínez, M., Pérez-Zabaleta, M., Quillaguamán, J., Larsson, G., 2015. Cultivation strategies for production of (R)-3-hydroxybutyric acid from simultaneous consumption of glucose, xylose and arabinose by Escherichia coli. Microb. Cell Fact. https://doi.org/10.1186/s12934-015-0236-2.
Jiang, J., Li, L., Li, Y., He, Y., Wang, C., Sun, Y., 2020. Bioaugmentation to enhance anaerobic digestion of food waste: Dosage, frequency and economic analysis. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2020.123256.
Jiang, X.R., Chen, G.Q., 2016. Morphology engineering of bacteria for bio-production. Biotechnol. Adv. https://doi.org/10.1016/j.biotechadv.2015.12.007.
Jiang, Y., Chen, Y., Zheng, X., 2009. Efficient polyhydroxyalkanoates production from a waste-activated sludge alkaline fermentation liquid by activated sludge submitted to the aerobic feeding and discharge process. Environ. Sci. Technol. https://doi.org/10.1021/es9014458.
Jiang, Y., Dimitry, Y., Kleerebezem, R., Muyzer, G., van Loosdrecht, M., 2011a. Plasticicumulans acidivorans gen. nov., sp. nov., a polyhydroxyalkanoate-accumulating gammaproteobacterium from a sequencing-batch bioreactor. Int. J. Syst. Evol. Microbiol. https://doi.org/10.1099/ijs.0.021410-0.
Jiang, Y., Hebly, M., Kleerebezem, R., Muyzer, G., van Loosdrecht, M.C.M., 2011b. Metabolic modeling of mixed substrate uptake for polyhydroxyalkanoate (PHA) production. Water Res. 45, 1309–1321. https://doi.org/10.1016/j.watres.2010.10.009.
Johnson, K., Jiang, Y., Kleerebezem, R., Muyzer, G., Van Loosdrecht, M.C.M., 2009. Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity, in: Biomacromolecules. https://doi.org/10.1021/bm8013796
Johnson, K., Kleerebezem, R., van Loosdrecht, M.C.M., 2010. Influence of the C/N ratio on the performance of polyhydroxybutyrate (PHB) producing sequencing batch reactors at short SRTs. Water Res. 44, 2141–2152. https://doi.org/10.1016/j.watres.2009.12.031.
Johnston, B., Radecka, I., Chiellini, E., Barsi, D., Ilieva, V.I., Sikorska, W., Musiol, M., Zięba, M., Chaber, P., Marek, A.A., Mendrek, B., Ekere, A.I., Adamus, G., Kowalczuk, M., 2019. Mass spectrometry reveals molecular structure of polyhydroxyalkanoates attained by bioconversion of oxidized polypropylene waste fragments. Polymers (Basel). https://doi.org/10.3390/polym11101580.
Kang, Z., Du, L., Kang, J., Wang, Y., Wang, Q., Liang, Q., Qi, Q., 2011. Production of succinate and polyhydroxyalkanoate from substrate mixture by metabolically engineered Escherichia coli. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2011.03.070.
Karmee, S.K., 2018. A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. Waste Manag. https://doi.org/10.1016/j.wasman.2017.10.042.
Khanna, S., Srivastava, A.K., 2005. Recent advances in microbial polyhydroxyalkanoates. Process Biochem. 40, 607–619. https://doi.org/10.1016/j.procbio.2004.01.053.
Kim, D.Y., Hyung, W.K., Moon, G.C., Young, H.R., 2007. Biosynthesis, modification, and biodegradation of bacterial medium-chain-length polyhydroxyalkanoates. J. Microbiol. 45, 87–97.
Koller, M., 2015. Recycling of waste streams of the biotechnological poly (hydroxyalkanoate) production by Haloferax mediterranei on whey. Int. J. Polym. Sci. 2015. https://doi.org/10.1155/2015/370164.
Koller, M., Bona, R., Chiellini, E., Fernandes, E.G., Horvat, P., Kutschera, C., Hesse, P., Braunegg, G., 2008. Polyhydroxyalkanoate production from whey by Pseudomonas hydrogenovora. Bioresour. Technol. 99, 4854–4863. https://doi.org/10.1016/j.biortech.2007.09.049.
Koller, M., Maršálek, L., de Sousa Dias, M.M., Braunegg, G., 2017. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. N. Biotechnol. https://doi.org/10.1016/j.nbt.2016.05.001.
Koller, M., Niebelschütz, H., Braunegg, G., 2013. Strategies for recovery and purification of poly[(R)-3-hydroxyalkanoates] (PHA) biopolyesters from surrounding biomass. Eng. Life Sci. https://doi.org/10.1002/elsc.201300021.
Kosseva, M.R., Rusbandi, E., 2018. Trends in the biomanufacture of polyhydroxyalkanoates with focus on downstream processing. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2017.09.054.
Kourmentza, C., Kornaros, M., 2016. Biotransformation of volatile fatty acids to polyhydroxyalkanoates by employing mixed microbial consortia: The effect of pH and carbon source. Bioresour. Technol. 222, 388–398. https://doi.org/10.1016/j.biortech.2016.10.014.
Kourmentza, C., Ntaikou, I., Lyberatos, G., Kornaros, M., 2015. Polyhydroxyalkanoates from Pseudomonas sp. using synthetic and olive mill wastewater under limiting conditions. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2014.12.032.
Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H. N., Reis, M.A.M., 2017. Recent Advances and Challenges towards Sustainable Polyhydroxyalkanoate (PHA) Production. Bioengineering 4, 55. https://doi.org/10.3390/bioengineering4020055.
Kovalcik, A., Kucera, D., Matouskova, P., Pernicova, I., Obruca, S., Kalina, M., Enev, V., Marova, I., 2018. Influence of removal of microbial inhibitors on PHA production from spent coffee grounds employing Halomonas halophila. J. Environ. Chem. Eng. https://doi.org/10.1016/j.jece.2018.05.028.
Kunasundari, B., Arza, C.R., Maurer, F.H.J., Murugaiyah, V., Kaur, G., Sudesh, K., 2017. Biological recovery and properties of poly(3-hydroxybutyrate) from Cupriavidus necator H16. Sep. Purif. Technol. 172, 1–6. https://doi.org/10.1016/j.seppur.2016.07.043.
Kunasundari, B., Sudesh, K., 2011. Isolation and recovery of microbial polyhydroxyalkanoates. Express Polym. Lett. 5, 620–634. https://doi.org/10.3144/expresspolymlett.2011.60.
Lageveen, R.G., Huisman, G.W., Preusting, H., Ketelaar, P., Eggink, G., Witholt, B., 1988. Formation of Polyesters by Pseudomonas oleovorans: Effect of Substrates on Formation and Composition of Poly-(R)-3-Hydroxyalkanoates and Poly-(R)-3-Hydroxyalkenoates. Appl. Environ. Microbiol. 54, 2924–2932. https://doi.org/10.1128/aem.54.12.2924-2932.1988.
Larriba, O., Rovira-Cal, E., Juznic-Zonta, Z., Guisasola, A., Baeza, J.A., 2020. Evaluation of the integration of P recovery, polyhydroxyalkanoate production and short cut nitrogen removal in a mainstream wastewater treatment process. Water Res. https://doi.org/10.1016/j.watres.2020.115474.
Lee, I.Y., Kim, G.J., Choi, D.K., Yeon, B.K., Park, Y.H., 1996. Improvement of Hydroxyvalerate Fraction in Poly(β-HydroxybutyrateCo-β-Hydroxyvalerate) by a Mutant Strain of Alcalisenes eutrophus. J. Ferment. Bioeng. https://doi.org/10.1016/0922-338X(96)82217-3.
Lee, J.W., Na, D., Park, J.M., Lee, J., Choi, S., Lee, S.Y., 2012. Systems metabolic engineering of microorganisms for natural and non-natural chemicals. Nat. Chem. Biol. https://doi.org/10.1038/nchembio.970.
Lemoigne, M., 1926. Produits de dehydration et de polymerisation de l'acide ß-oxobutyrique. Bull. Soc. Chim. Biol. (Paris) 8, 770–778.
Li, M., Wilkins, M., 2020. Fed-batch cultivation and adding supplements to increase yields of polyhydroxybutyrate production by Cupriavidus necator from corn stover alkaline pretreatment liquor. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.122676.
Li, Z.J., Cai, L., Wu, Q., Chen, G.Q., 2009. Overexpression of NAD kinase in recombinant Escherichia coli harboring the phbCAB operon improves poly(3-hydroxybutyrate) production. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-009-1943-6.
Liebergesell, M., Steinbüchel, A., 1992. Cloning and nucleotide sequences of genes relevant for biosynthesis of poly(3-hydroxybutyric acid) in Chromatium vinosum strain D. Eur. J. Biochem. https://doi.org/10.1111/j.1432-1033.1992.tb17270.x.
Lim, S.-J., Jung, Y.-M., Shin, H.-D., Lee, Y.-H., 2002. Amplification of the NADPH-Related Genes zwf and gnd for the Oddball Biosynthesis of PHB in an E. coli Transformant Harboring a Cloned phb CAB Operon. J. Biosci. Bioeng. https://doi.org/10.1263/jbb.93.543.
López-Abelairas, M., García-Torreiro, M., Lú-Chau, T., Lema, J.M., Steinbüchel, A., 2015. Comparison of several methods for the separation of poly(3-hydroxybutyrate) from Cupriavidus necator H16 cultures. Biochem. Eng. J. https://doi.org/10.1016/j.bej.2014.10.018.
Lu, X., Liu, Yuwan, Yang, Y., Wang, S., Wang, Q., Wang, X., Yan, Z., Cheng, J., Liu, C., Yang, X., Luo, H., Yang, S., Gou, J., Ye, L., Lu, L., Zhang, Z., Guo, Y., Nie, Y., Lin, J., Li, S., Tian, C., Cai, T., Zhuo, B., Ma, H., Wang, W., Ma, Y., Liu, Yongjun, Li, Y., Jiang, H., 2019. Constructing a synthetic pathway for acetyl-coenzyme A from one-carbon through enzyme design. Nat. Commun. https://doi.org/10.1038/s41467-019-09095-z.
Lv, L., Ren, Y.-L., Chen, J.-C., Wu, Q., Chen, G.-Q., 2015. Application of CRISPRi for prokaryotic metabolic engineering involving multiple genes, a case study: Controllable P(3HB-co-4HB) biosynthesis. Metab. Eng. 29, 160–168. https://doi.org/10.1016/j.ymben.2015.03.013.
Madison, L.L., Huisman, G.W., 1999. Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. Microbiol. Mol. Biol. Rev.

Maier, R.M., Gentry, T.J., 2014. Microorganisms and Organic Pollutants, in: Environmental Microbiology: Third Edition. https://doi.org/10.1016/B978-0-12-394626-3.00017-X

Mannina, G., Presti, D., Montiel-Jarillo, G., Suárez-Ojeda, M.E., 2019. Bioplastic recovery from wastewater: A new protocol for polyhydroxyalkanoates (PHA) extraction from mixed microbial cultures. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.03.037.

Marang, L., Jiang, Y., van Loosdrecht, M.C.M., Kleerebezem, R., 2013. Butyrate as preferred substrate for polyhydroxybutyrate production. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2013.05.031.

Marang, L., van Loosdrecht, M.C.M., Kleerebezem, R., 2018. Enrichment of PHA-producing bacteria under continuous substrate supply. N. Biotechnol. https://doi.org/10.1016/j.nbt.2017.12.001.

Mendonça, T.T., Gomez, J.G.C., Buffoni, E., Sánchez Rodriguez, R.J., Schripsema, J., Lopes, M.S.G., Silva, L.F., 2014. Exploring the potential of Burkholderia sacchari to produce polyhydroxyalkanoates. J. Appl. Microbiol. https://doi.org/10.1111/jam.12406.

Mohamad Fauzi, A.H., Chua, A.S.M., Yoon, L.W., Nittami, T., Yeoh, H.K., 2019. Enrichment of PHA-accumulators for sustainable PHA production from crude glycerol. Process Saf. Environ. Prot. https://doi.org/10.1016/j.psep.2018.12.002.

Mohammadi, M., Hassan, M.A., Phang, L.Y., Shirai, Y., Man, H.C., Ariffin, H., 2012. Intracellular polyhydroxyalkanoates recovery by cleaner halogen-free methods towards zero emission in the palm oil mill. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2012.07.038.

Montiel-Jarillo, G., Carrera, J., Suárez-Ojeda, M.E., 2017. Enrichment of a mixed microbial culture for polyhydroxyalkanoates production: Effect of pH and N and P concentrations. Sci. Total Environ. 583, 300–307. https://doi.org/10.1016/j.scitotenv.2017.01.069.

Morgan-Sagastume, F., Hjort, M., Cirne, D., Gérardin, F., Lacroix, S., Gaval, G., Karabegovic, L., Alexandersson, T., Johansson, P., Karlsson, A., Bengtsson, S., Arcos-Hernández, M.V., Magnusson, P., Werker, A., 2015. Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2015.01.046.

Morya, R., Kumar, M., Thakur, I.S., 2018. Utilization of glycerol by Bacillus sp. ISTVK1 for production and characterization of Polyhydroxyvalerate. Bioresour. Technol. Reports. https://doi.org/10.1016/j.biteb.2018.03.002.

Możejko-Ciesielska, J., Kiewisz, R., 2016. Bacterial polyhydroxyalkanoates: Still fabulous? Microbiol. Res. https://doi.org/10.1016/j.micres.2016.07.010.

Muhammadi, Shabina, Afzal, M., Hameed, S., 2015. Bacterial polyhydroxyalkanoates-eco-friendly next generation plastic: Production, biocompatibility, biodegradation, physical properties and applications. Green Chem. Lett. Rev. https://doi.org/10.1080/17518253.2015.1109715.

Murugan, P., Han, L., Gan, C.-Y., Maurer, F.H.J., Sudesh, K., 2016. A new biological recovery approach for PHA using mealworm, Tenebrio molitor. J. Biotechnol. 239, 98–105. https://doi.org/10.1016/j.jbiotec.2016.10.012.

Nguyen, R.T., Fishman, T., Zhao, F., Imholte, D.D., Graedel, T.E., 2018. Analyzing critical material demand: A revised approach. Sci. Total Environ. 630, 1143–1148. https://doi.org/10.1016/j.scitotenv.2018.02.283.

Nielsen, C., Rahman, A., Rehman, A.U., Walsh, M.K., Miller, C.D., 2017. Food waste conversion to microbial polyhydroxyalkanoates. Microb. Biotechnol. 10, 1338–1352.

Noorman, H.J., Heijnen, J.J., 2017. Biochemical engineering's grand adventure. Chem. Eng. Sci. https://doi.org/10.1016/j.ces.2016.12.065.

Obruca, Stanislav, Benesova, P., Kucera, D., Petrik, S., Marova, I., 2015a. Biotechnological conversion of spent coffee grounds into polyhydroxyalkanoates and carotenoids. N. Biotechnol. https://doi.org/10.1016/j.nbt.2015.02.008.

Obruca, S., Benesova, P., Marsalek, L., Marova, I., 2015b. Use of lignocellulosic materials for PHA production. Chem. Biochem. Eng. Q. https://doi.org/10.15255/CABEQ.2014.2253.

Obruca, S., Benesova, P., Petrik, S., Oborna, J., Prikryl, R., Marova, I., 2014a. Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds. Process Biochem. https://doi.org/10.1016/j.procbio.2014.05.013.

Obruca, S., Petrik, S., Benesova, P., Svoboda, Z., Eremka, L., Marova, I., 2014b. Utilization of oil extracted from spent coffee grounds for sustainable production of polyhydroxyalkanoates. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-014-5653-3.

Obruca, S., Petrik, S., Benesova, P., Svoboda, Z., Eremka, L., Marova, I., 2014c. Utilization of oil extracted from spent coffee grounds for sustainable production of polyhydroxyalkanoates. Appl. Microbiol. Biotechnol. 98, 5883–5890. https://doi.org/10.1007/s00253-014-5653-3.

Obruca, S., Marova, I., Snajdar, O., Mravcova, L., Svoboda, Z., 2010. Production of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by Cupriavidus necator from waste rapeseed oil using propanol as a precursor of 3-hydroxyvalerate. Biotechnol. Lett. https://doi.org/10.1007/s10529-010-0376-8

Oever, M. van den, Molenveld, K., Zee, M. van der, Bos, H., 2017. Bio-based and biodegradable plastics : facts and figures : focus on food packaging in the Netherlands, Wageningen Food & Biobased Research : 1722. Wageningen Food & Biobased Research, 714, ,.

Padan, E., Bibi, E., Ito, M., Krulwich, T.A., 2005. Alkaline pH homeostasis in bacteria: New insights. Biochim. Biophys. Acta - Biomembr. 1717, 67–88. https://doi.org/10.1016/j.bbamem.2005.09.010.

Page, W.J., Knosp, O., 1989. Hyperproduction of poly-β-hydroxybutyrate during exponential growth of Azotobacter vinelandii UWD. Appl. Environ. Microbiol.

Pais, J., Serafim, L.S., Freitas, F., Reis, M.A.M., 2016. Conversion of cheese whey into poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by Haloferax mediterranei. N. Biotechnol. 33, 224–230. https://doi.org/10.1016/j.nbt.2015.06.001.

Passanha, P., Esteves, S.R., Kedia, G., Dinsdale, R.M., Guwy, A.J., 2013. Increasing polyhydroxyalkanoate (PHA) yields from Cupriavidus necator by using filtered digestate liquors. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2013.08.050.

Patel, M., Gapes, D.J., Newman, R.H., Dare, P.H., 2009. Physico-chemical properties of polyhydroxyalkanoate produced by mixed-culture nitrogen-fixing bacteria. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-008-1836-0.

Perez-Zabaleta, M., Guevara-Martínez, M., Gustavsson, M., Quillaguamán, J., Larsson, G., van Maris, A.J.A., 2019. Comparison of engineered Escherichia coli AF1000 and BL21 strains for (R)-3-hydroxybutyrate production in fed-batch cultivation. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-019-09876-y.

Perez-Zabaleta, M., Sjöberg, G., Guevara-Martínez, M., Jarmander, J., Gustavsson, M., Quillaguamán, J., Larsson, G., 2016. Increasing the production of (R)-3-hydroxybutyrate in recombinant Escherichia coli by improved cofactor supply. Microb. Cell Fact. https://doi.org/10.1186/s12934-016-0490-y.

Pernicova, I., Kucera, D., Nebesarova, J., Kalina, M., Novackova, I., Koller, M., Obruca, S., 2019. Production of polyhydroxyalkanoates on waste frying oil employing selected Halomonas strains. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.122028.

Pescuma, M., de Valdez, G.F., Mozzi, F., 2015. Whey-derived valuable products obtained by microbial fermentation. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-015-6766-z.

Plastics Europe, 2019. Plastics- the Facts 2019. Plastics Europe Deutschland, Düsseldorf.

Rahman, A., Linton, E., Hatch, A.D., Sims, R.C., Miller, C.D., 2013. Secretion of polyhydroxybutyrate in Escherichia coli using a synthetic biological engineering approach. J. Biol. Eng. 7, 24. https://doi.org/10.1186/1754-1611-7-24.

Raza, Z.A., Abid, S., Banat, I.M., 2018. Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. Int. Biodeterior. Biodegrad. https://doi.org/10.1016/j.ibiod.2017.10.001.

Ren, Y., Ling, C., Hajnal, I., Wu, Q., Chen, G.Q., 2018. Construction of Halomonas bluephagenesis capable of high cell density growth for efficient PHA production. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-018-8931-7.

Rodriguez-Perez, S., Serrano, A., Pantión, A.A., Alonso-Fariñas, B., 2018. Challenges of scaling-up PHA production from waste streams. A review. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2017.09.083.

Rosengart, A., Cesário, M.T., de Almeida, M.C.M.D., Raposo, R.S., Espert, A., de Apodaca, E.D., da Fonseca, M.M.R., 2015. Efficient P(3HB) extraction from Burkholderia sacchari cells using non-chlorinated solvents. Biochem. Eng. J. 103, 39–46. https://doi.org/10.1016/j.bej.2015.06.013.

Samorì, C., Abbondanzi, F., Galletti, P., Giorgini, L., Mazzocchetti, L., Torri, C., Tagliavini, E., 2015. Extraction of polyhydroxyalkanoates from mixed microbial cultures: Impact on polymer quality and recovery. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2015.03.062.

Sangkharak, K., Khaithongkaeo, P., Chuaikhunupakarn, T., Choonut, A., Prasertsan, P., 2020. The production of polyhydroxyalkanoate from waste cooking oil and its application in biofuel production. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-020-00657-6.

Schlegel, H.G., Gottschalk, G., Von Bartha, R., 1961. Formation and utilization of poly-β-hydroxybutyric acid by knallgas bacteria (hydrogenomonas). Nature. https://doi.org/10.1038/191463a0.

Schmidt, M., Ienczak, J.L., Quines, L.K., Zanfonato, K., Schmidell, W., de Aragão, G.M.F., 2016. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) production in a system with external cell recycle and limited nitrogen feeding during the production phase. Biochem. Eng. J. 112, 130–135. https://doi.org/10.1016/j.bej.2016.04.013.

Serafim, L.S., Lemos, P.C., Albuquerque, M.G.E., Reis, M.A.M., 2008. Strategies for PHA production by mixed cultures and renewable waste materials. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-008-1757-y.

Shah, A.A., Hasan, F., Hameed, A., Ahmed, S., 2008. Biological degradation of plastics: A comprehensive review. Biotechnol. Adv. 26, 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005.

Sharma, P.K., Munir, R.I., Blunt, W., Dartiailh, C., Cheng, J., Charles, T.C., Levin, D.B., 2017. Synthesis and physical properties of polyhydroxyalkanoate polymers with different monomer compositions by recombinant Pseudomonas putida LS46 expressing a novel PHA SYNTHASE (PhaC116) enzyme. Appl. Sci. https://doi.org/10.3390/app7030242.

Shen, R., Yin, J., Ye, J.W., Xiang, R.J., Ning, Z.Y., Huang, W.Z., Chen, G.Q., 2018. Promoter Engineering for Enhanced P(3HB- co-4HB) Production by Halomonas bluephagenesis. ACS Synth. Biol. https://doi.org/10.1021/acssynbio.8b00102.

Silva, F., Campanari, S., Matteo, S., Valentino, F., Majone, M., Villano, M., 2017. Impact of nitrogen feeding regulation on polyhydroxyalkanoates production by mixed microbial cultures. N. Biotechnol. 37, 90–98. https://doi.org/10.1016/j.nbt.2016.07.013.

Snell, K.D., Feng, F., Zhong, L., Martin, D., Madison, L.L., 2002. YfcX enables medium-chain-length poly(3-hydroxyalkanoate) formation from fatty acids in recombinant Escherichia coli fadB strains. J. Bacteriol. https://doi.org/10.1128/JB.184.20.5696-5705.2002.

Son, H., Park, G., Lee, S., 1996. Growth-associated production of poly-β-hydroxybutyrate from glucose or alcoholic distillery wastewater by Actinobacillus sp. EL-9. Biotechnol. Lett. https://doi.org/10.1007/BF00129945

Stal, L.J., 1992. Poly(hydroxyalkanoate) in cyanobacteria: an overview. FEMS Microbiol. Lett. https://doi.org/10.1016/0378-1097(92)90307-A.

Sudesh, K., Abe, H., Doi, Y., 2000. Synthesis, structure and properties of polyhydroxyalkanoates: Biological polyesters. Prog. Polym. Sci. https://doi.org/10.1016/S0079-6700(00)00035-6.

Sun, Z., Ramsay, J.A., Guay, M., Ramsay, B.A., 2007. Fermentation process development for the production of medium-chain-length poly-3-hyroxyalkanoates. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-007-0857-4.

Tao, W., Lv, L., Chen, G.Q., 2017. Engineering Halomonas species TD01 for enhanced polyhydroxyalkanoates synthesis via CRISPRi. Microb. Cell Fact. https://doi.org/10.1186/s12934-017-0655-3.

Taran, M., Azizi, E., Taran, S., Asadi, N., 2011. Archaeal Poly (3-hydroxybutyrate) Polymer Production from Glycerol: Optimization by Taguchi Methodology. J. Polym. Environ. 19, 750–754. https://doi.org/10.1007/s10924-011-0327-z.

Tian, H., Yan, M., Treu, L., Angelidaki, I., Fotidis, I.A., 2019. Hydrogenotrophic methanogens are the key for a successful bioaugmentation to alleviate ammonia inhibition in thermophilic anaerobic digesters. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.122070.

Tomei, M.C., Bertanza, G., Canato, M., Heimersson, S., Laera, G., Svanström, M., 2016. Techno-economic and environmental assessment of upgrading alternatives for sludge stabilization in municipal wastewater treatment plants. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2015.10.017.

Valdés, J., Kutralam-Muniasamy, G., Vergara-Porras, B., Marsch, R., Pérez-Guevara, F., López-Cuellar, M.R., 2018. Heterologous expression of phaC2 gene and poly-3-hydroxyalkanoate production by recombinant Cupriavidus necator strains using canola oil as carbon source. N. Biotechnol. https://doi.org/10.1016/j.nbt.2017.08.001.

Valentino, F., Moretto, G., Lorini, L., Bolzonella, D., Pavan, P., Majone, M., 2019. Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge. Ind. Eng. Chem. Res. https://doi.org/10.1021/acs.iecr.9b01831.

Valentino, F., Morgan-Sagastume, F., Campanari, S., Villano, M., Werker, A., Majone, M., 2017. Carbon recovery from wastewater through bioconversion into biodegradable polymers. N. Biotechnol. https://doi.org/10.1016/j.nbt.2016.05.007.

Venkata Mohan, S., Nikhil, G.N., Chiranjeevi, P., Nagendranatha Reddy, C., Rohit, M. V., Kumar, A.N., Sarkar, O., 2016. Waste biorefinery models towards sustainable circular bioeconomy: Critical review and future perspectives. Bioresour. Technol. 215, 2–12. https://doi.org/10.1016/j.biortech.2016.03.130.

Villano, M., Beccari, M., Dionisi, D., Lampis, S., Miccheli, A., Vallini, G., Majone, M., 2010. Effect of pH on the production of bacterial polyhydroxyalkanoates by mixed cultures enriched under periodic feeding. Process Biochem. 45, 714–723. https://doi.org/10.1016/j.procbio.2010.01.008.

Villano, M., Valentino, F., Barbetta, A., Martino, L., Scandola, M., Majone, M., 2014. Polyhydroxyalkanoates production with mixed microbial cultures: From culture selection to polymer recovery in a high-rate continuous process. N. Biotechnol. 31, 289–296. https://doi.org/10.1016/j.nbt.2013.08.001.

Wang, S., Chen, W., Xiang, H., Yang, J., Zhou, Z., Zhu, M., 2016. Modification and potential application of short-chain-length polyhydroxyalkanoate (SCL-PHA). Polymers (Basel). https://doi.org/10.3390/polym8080273.

Wang, X., Oehmen, A., Freitas, E.B., Carvalho, G., Reis, M.A.M., 2017. The link of feast-phase dissolved oxygen (DO) with substrate competition and microbial selection in PHA production. Water Res. https://doi.org/10.1016/j.watres.2017.01.064.

Wang, Y., Wu, H., Jiang, X., Chen, G.Q., 2014. Engineering Escherichia coli for enhanced production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) in larger cellular space. Metab. Eng. https://doi.org/10.1016/j.ymben.2014.07.010.

Wang, Y., Yin, J., Chen, G.-Q., 2014b. Polyhydroxyalkanoates, challenges and opportunities. https://doi.org/10.1016/j.copbio.2014.06.001

Weiss, T.L., Young, E.J., Ducat, D.C., 2017. A synthetic, light-driven consortium of cyanobacteria and heterotrophic bacteria enables stable polyhydroxybutyrate production. Metab. Eng. https://doi.org/10.1016/j.ymben.2017.10.009.

Wen, Q., Chen, Z., Tian, T., Chen, W., 2010. Effects of phosphorus and nitrogen limitation on PHA production in activated sludge. J. Environ. Sci. https://doi.org/10.1016/S1001-0742(09)60295-3.

Werker, A., Bengtsson, S., Korving, L., Hjort, M., Anterrieu, S., Alexandersson, T., Johansson, P., Karlsson, A., Karabegovic, L., Magnusson, P., Morgan-Sagastume, F., Sijstermans, L., Tietema, M., Visser, C., Wypkema, E., Van Der Kooij, Y., Deeke, A., Uijterlinde, C., 2018. Consistent production of high quality PHA using activated sludge harvested from full scale municipal wastewater treatment - PHARIO. Water Sci. Technol. https://doi.org/10.2166/wst.2018.502.

Yadav, D., Singh, N.K., Pruthi, V., Kumar, P., 2020. Ensuring sustainability of conventional aerobic wastewater treatment system via bio-augmentation of aerobic bacterial consortium: An enhanced biological phosphorus removal approach. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2020.121328.

Yin, F., Li, D., Ma, X., Zhang, C., 2019. Pretreatment of lignocellulosic feedstock to produce fermentable sugars for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) production using activated sludge. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.121773.

Yu, J., Chen, L.X.L., 2006. Cost-effective recovery and purification of polyhydroxyalkanoates by selective dissolution of cell mass. Biotechnol. Prog. https://doi.org/10.1021/bp050362g.

Zainab-L, I., Sudesh, K., 2019. High cell density culture of Cupriavidus necator H16 and improved biological recovery of polyhydroxyalkanoates using mealworms. J. Biotechnol. https://doi.org/10.1016/j.jbiotec.2019.09.001.

Zhang, M., Kurita, S., Orita, I., Nakamura, S., Fukui, T., 2019. Modification of acetoacetyl-CoA reduction step in Ralstonia eutropha for biosynthesis of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) from structurally unrelated compounds. Microb. Cell Fact. https://doi.org/10.1186/s12934-019-1197-7.

Zhuang, Q., Qi, Q., 2019. Engineering the pathway in Escherichia coli for the synthesis of medium-chain-length polyhydroxyalkanoates consisting of both even- A nd odd-chain monomers. Microb. Cell Fact. https://doi.org/10.1186/s12934-019-1186-x.

Zinn, M., Witholt, B., Egli, T., 2001. Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. Adv. Drug Deliv. Rev. 53, 5–21. https://doi.org/10.1016/S0169-409X(01)00218-6.