The production of polyhydroxyalkanoates using volatile fatty acids derived from the acidogenic biohydrogen effluents: An overview

Patrick Sekoai [a,*], Obinna Ezeokoli [b], Kelvin Yoro [c], Orevaoghene Eterigho-Ikelegbe [d], Olivier Habimana [e], Samuel Iwarere [f], Michael Daramola [f], Tunde Ojumu [a]

[a]Department of Chemical Engineering, Cape Peninsula University of Technology, Bellville, Cape Town 7535, South Africa
[b]Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa
[c]Energy Technologies Area, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, United States
[d]DSI-NRF SARChI Clean Coal Technology Research Group, School of Chemical and Metallurgical Engineering, Faculty of Engineering and the Built Environment, University of the Witwatersrand, Wits 2050, Johannesburg, South Africa
[e]The School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region
[f]Department of Chemical Engineering, Faculty of Engineering, Built Environment and Information Technology, University of Pretoria, Hatfield 0028, South Africa

*Corresponding author. Email: patricksekoai@gmail.com

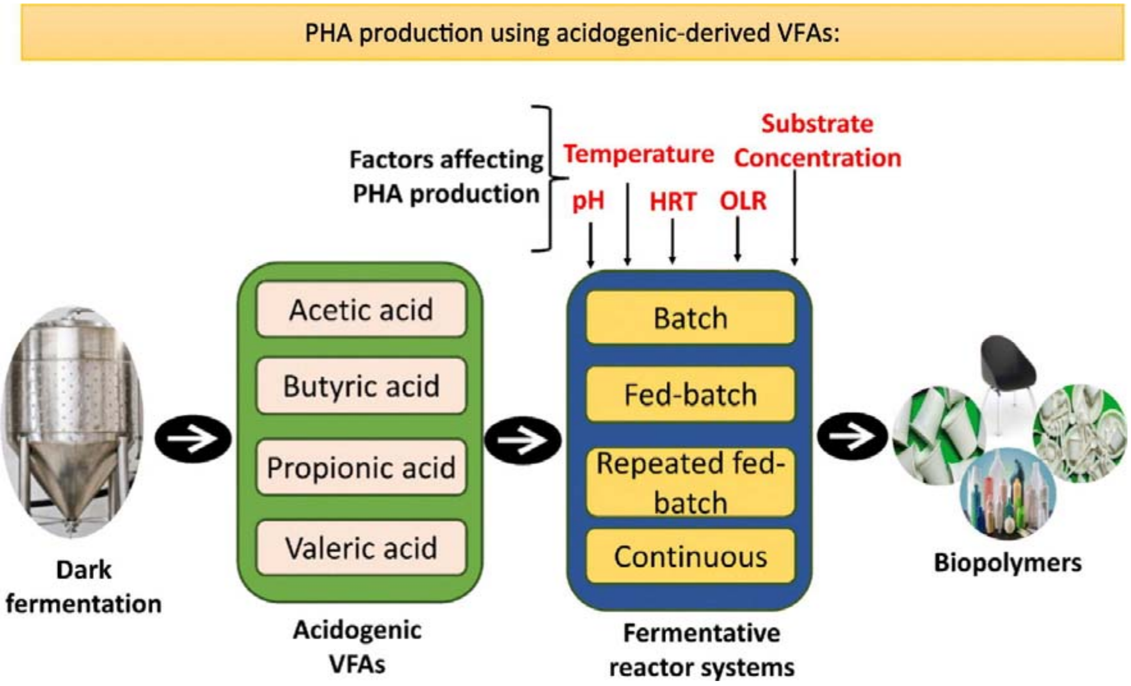
Highlights

- The production of PHAs using acidogenic-derived VFAs is explored in this paper.
- The operational parameters that contribute to the recovery of PHAs are discussed.
- The challenges that hinder the industrialization of PHAs are elucidated as well.
- The paper provides suggestions that could help surpass the current limitations.
-

Abstract

Biohydrogen production is regarded as the cleanest process of producing H_2 due to its non-polluting features and its ability to valorize wastes. However, its industrialization remains stagnant due to the process barriers facing this technology. Research is now geared towards the beneficiation of acidogenic-derived metabolites to improve its competitiveness. The acidogenic effluents consist of chemical precursors known as volatile fatty acids (VFAs), which can serve as cheap substrates for polyhydroxyalkanoates (PHAs). PHAs are emerging as a potential replacement for petroleum-based plastics due to their environmental friendliness, biodegradability, and cost-effectiveness. This overview discusses the synthesis of PHAs using acidogenic-derived VFAs as a carbon source. It examines the setpoint parameters that affect the recovery of PHAs using VFAs that are obtained from acidogenic effluents. Furthermore, the challenges that hinder the industrialization of PHAs are elucidated. The paper concludes by providing suggestions that could fast-track the development of PHAs using waste effluents.

Graphical abstract



Keywords: Acidogenic fermentation; Polyhydroxyalkanoates; Volatile fatty acids

1. Introduction

The pressing energy issues coupled with environmental deterioration have triggered a need to search for alternative energy solutions. Therefore, the United Nations has outlined a roadmap that will ensure that alternative energy needs are met by the year 2030 (Hughes and Jones, 2020). Therefore, many countries, including those that emit a lot of carbon emissions, are expected to produce at least 30% of alternative fuels in the energy sector and 10% of clean energy in the transport sector (Hughes and Jones, 2020). Amongst the existing energy technologies that are being investigated within the scientific community, biomass valorization targeting biofuels is part of the aforementioned goals and is gaining increasing popularity amongst researchers. These biomass-derived fuels are produced using technologies that are environmentally-friendly and affordable (Mhlongo et al., 2021; Sekoai et al., 2021).

Fermentative biohydrogen production is a biotechnological process that uses anaerobic microorganisms for the conversion of organic substrates into biohydrogen and volatile fatty acids (VFAs) (Sharmila et al., 2022). This process is considered attractive because of its potential role in renewable energy development (Pandey et al., 2022). However, the low bioH$_2$ yields hinder the scalability of this bioprocess due to the accumulation of intermediate by-products (mainly VFAs). This has triggered the development of other biotechnological methods that can be used to valorize/beneficiate these chemical precursors (Li et al., 2022).

VFAs are short-chain fatty acids consisting of C$_2$-C$_6$ atoms and these compounds are found in different environmental sites such as soils, water, and intestines of mammals (Pandey et al., 2022). In recent years, they have received widespread attention due to their applicability in numerous fields such as pharmaceuticals, chemicals, polymers, biofuels, and wastewater treatment (Sharmila et al., 2022). Their production is expected to increase in the upcoming years due to their diverse uses. For example, approximately 10 million tons of acetic acid is produced globally and this value is expected to reach 20 million tons in 2025 (Pal and Nayak, 2017). Likewise, the demand for butyric acid is also growing due to its application in medicine, animal feed, and food additives. The global production of butyric acid is estimated at 300000 tons and has an annual growth rate of 2.5% (Dwidar et al., 2012). Other VFAs such as lactic acid and propionic acid have numerous applications as well. The worldwide demand for lactic acid and propionic acid is presently estimated at 150000 and 400,000 tons, respectively (BCC Research, 2019). The global market for VFAs will grow from nearly $13.7 billion in 2018 to $18.0 billion by 2023 (BCC Research, 2019). The industrial production of VFAs is primarily achieved using chemical processes like the oxidation or carboxylation of precursors such as aldehyde and alkenes (Turon et al., 2016). Nonetheless, the high reliance on energy-intensive VFA-producing processes contribute to CO_2 emissions and environmental pollution. For this reason, scientists are now exploring the use of acidogenic-derived VFAs to make this technology economical, sustainable, and ecologically-friendly. The term "acidogenic-derived VFAs" was established by scientists to describe VFAs that have been obtained from the biohydrogen fermentation whereby microbes are used to synthesize mainly bioH_2 and VFAs.

Polyhydroxyalkanoates (PHAs) are emerging as a suitable replacement for petroleum-based plastics owing to their biodegradability, versatility, sustainability, and affordability (Surendran et al., 2020). These renewable polyesters are used in numerous applications, such as the food industry, medicine, agriculture, and cosmetics (Novelli et al., 2021). They are produced by a broad spectrum of microbial populations under aerobic and anaerobic microenvironments. However, scale-up studies in PHAs are still costly because the microbial strains operate under stringent bioprocess conditions (Kalia et al., 2021). As a consequence, research is now tailored towards finding alternative ways of synthesizing these biopolymers, particularly methods that are cheap and environmentally-friendly (Kalia et al., 2021). It has been shown that acidogenic-derived VFAs are desirable substrates in PHA production because they lower the operational costs since these intermediates are considered as waste and are easily accessible (Jayakrishnan et al., 2021). Therefore, the acidogenic-derived effluents which consist of VFAs could play a crucial role in the biotechnological advancement of PHAs. Research shows that >50% of these intermediates remain in the bioH_2 spent medium (Banu et al., 2021), implying that they could serve as a valuable carbon source for PHA-producers.

1.1. Importance of study and contribution to knowledge

Novel methods are presently being explored to reinvigorate interest in biohydrogen production and advance its development as an alternative fuel. Attention is now directed towards the beneficiation/valorization of acidogenic effluents into valuable compounds such as PHAs to enhance the energetic gains and economic value of this process. The acidogenic-derived VFAs consist of functional groups that are applicable in chemicals, plastics, biofuels, and wastewater treatment. Although the use of these VFAs in PHA production has been proposed in the literature (Khatami et al., 2022; Lagoa-Costa et al., 2022; Rajendran and Han, 2022), few published studies have covered this topic, particularly in the form of a review article, implying that there is a dearth of knowledge regarding the use of acidogenic VFAs in PHAs. Therefore, this paper will examine the use of acidogenic-derived VFAs as precursors/substrates for PHA production. This work will also explore the operational parameters affecting the accumulation of PHAs. Finally, the review will discuss the current challenges and future directions in the synthesis of PHAs using VFAs from acidogenic or waste effluents.

2. What are polyhydroxyalkanoates (PHAs)?

PHAs are groups of polymeric esters that are synthesized by a broad spectrum of microbial communities. Up until now, >300 microbial cell factories can produce over 100 types of these biopolymers (Kumar et al., 2019; Jayakrishnan et al., 2021). They accumulate within the cells of microbial species and have a diameter that ranges from 0.2 to 0.5 µm. Such biopolymers can assume different morphological structures (brittle thermoplastic or gummy elastomers) depending on bioprocess conditions (Raza et al., 2018). PHAs are classified based on their chain-lengths, and these are (i) short chain-lengths (scl-PHAs), (ii) medium chain-lengths (mcl-PHAs), and (iii) long-chain lengths (lcl-PHA) (Jayakrishnan et al., 2021).

Amongst these, scl-PHAs are widely investigated in the literature. They have been shown to have industrial relevance, although they possess weak structural stability in comparison to mcl-PHAs, as these polyesters tend to be rubbery and elastic (Wang et al., 2016). Besides, the well-known scl-PHAs that are covered in the literature include poly(3-hydroxyvalerate), poly(3-hydroxybutyrate), and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (McAdam et al., 2020). Owing to their exquisite properties such as biocompatibility, tuneable structure, and biodegradability, PHAs will play an important role in the advancement of bio-based plastics and biomaterials in the next upcoming years (Vu et al., 2021).

3. The production of PHAs using acidogenic-derived VFAs

To fast-track the industrialization of PHAs, it is imperative to reduce the production costs and one of the key factors that will be instrumental in reducing these costs is the exploitation of inexpensive carbon sources as mentioned earlier (Banu et al., 2021). Over the past decade, research has been directed towards the intensification of biosynthetic routes that use wastes alongside cheap inoculums (MMC) during PHA production as shown in Section 5.2 of this review. As a consequence, various acidogenic streams originating from lignocellulosic, agro-municipal, and industrial wastes have been successfully used in the biosynthesis of PHAs (Khatami et al., 2021), and some of these studies will be covered in this Section.

Although the use of solid wastes presents a lot of merits in microbial production of PHAs, these carbon materials must undergo preliminary pretreatment steps before being used in PHA production, and will further escalate the manufacturing costs. To improve the competitiveness of microbial synthesis of PHAs, innovative methods must be explored in the literature to pave the way for the scalability of this process (Saratale et al., 2021).

The valorization of acidogenic effluents (fermented liquids) has garnered increasing attention amongst scientists owing to the fact that these spent liquids are rich in VFAs and these can serve as suitable substrates for PHA-accumulating species (Table 1). They provide an economic approach to producing PHAs as the acidogenic step is not needed since the effluents are directly obtained from acidogenic fermentation during the concomitant production of biohydrogen and VFAs as discussed in Section 1, and are considered to be waste (Woo and Kim, 2019). Besides, these chemical precursors consist of functional groups which allow them to be used in numerous applications including cosmetics, chemicals, biofuels, beverages, and pharmaceuticals (Baumann and Westermann, 2016; Kumar et al., 2019), as stated earlier.

Table 1. The production of PHAs using VFAs derived from the acidogenic effluents.

Type of effluents	VFAs found in the effluents	Inoculum source	Reactor type	Operational conditions	PHA content (%)	PHA type	PHA yield	Reference
Fermented waste	Acetic acid, butyric acid, valeric acid, propionic acid	Mixed cultures	Fed-batch	pH = 7.0, Temp = 29 °C,	36.9	3HB, 3 HV	–	Reddy and Mohan (2012)
Co-digested waste streams	Butyric acid, propionic acid, isovaleric acid	Mixed cultures	Fed-batch	pH = 8.0, Temp = 30 °C	32.5	3HB, 3HH, 3 HV	–	Iglesias-Iglesias et al. (2021)
Starchy wastewater	Acetic acid, propionic acid, butyric acid,	*Alcaligenes eutrophus*	Fed-batch	pH = 11.0, Temp = 30 °C	48	3HB, 3 HV	1.0–1.3 g/L	Yu (2001)
Paper mill wastewater	Acetic acid, propionic acid, butyric acid, valeric acid	Mixed cultures	Batch	pH = 7.3, Temp = 30 °C	48	3HB, 3 HV	0.26–0.40 C-mol/C-mol	Bengtsson et al. (2008b)
Potato peels	Acetic acid, butyric acid, propionic acid	*Pseudomonas oleovorans*	Batch	pH = 7.0, Temp = 30 °C,	39	–	0.39 g PHAs/g DCW	Aremu et al. (2021)
Food waste	Acetic acid, propionic acid, butyric acid, isovaleric acid, hexanoic acid	*Cupriavidus necator*	Batch	pH = 7.0, Temp = 30 °C	77	3HB	1.723 g/L	Khatami et al. (2022)
Cheese whey	Acetic acid, butyric acid, Propionic acid, lactic acid, valeric acid	Mixed cultures	Fed-batch	pH = 8.0–8.5, Temp = 30 °C	45–50	3HB, 3 HV	–	Lagoa-Costa et al. (2022)
Brewer's spent grain	Acetic acid	*Burkholderia cepacian*	Fed-batch	pH 5.5, Temp = 30 °C	–	3HB, 3 HV	13.1 mg PHA/g BSG	Martínez-Avila et al. (2022)
Fermented effluents	Acetic acid, butyric acid, propionic acid	*Halomonas boliviensis*	Fed-batch	pH 7.5, Temp = 30 °C	70	3HB-co-3 HV	13.0 g/L	García-Torreiro et al. (2016)
Organic wastes	Acetic acid, propionic acid	Mixed culture	Fed-batch	No pH control, Temp = 24 °C	71	3HB, 3 HV	0.20–0.67 C-mmol/C-mmol	Vargas et al. (2014)
Fermented waste	Acetic acid, butyric acid, propionic acid	*Pseudomonas otitidis*	Batch	pH = 7.0, Temp = 37 °C	58	3HB-co-3 HV	–	Reddy et al. (2012)
Fermented waste	Acetic acid, propionic acid, valeric acid	*Bacillus tequilensis*	Batch	pH = 7.0, Temp = 29 °C	40	3HB-co-3 HV	7.8 g/L	Amulya et al. (2014)
Fermented waste	Acetic acid, butyric acid	*Cupriavidus necator*	Batch	pH = 7.2, Temp = 30 °C	63	3HB	0.25–0.27 g PHA/g VFAs	Martinez et al. (2016)

Cheese whey	Hexanoic acid, octanoic acid	*Cupriavidus necator*	Fed-batch	pH = 7.0, Temp = 30 °C	71	3HB	0.60 g PHAs/g VFAs	Domingos et al. (2018)
Dairy wastewater	Acetic acid, butyric acid, propionic acid	*Cupriavidus necator*	Fed-batch	pH = 6.5, Temp = 30 °C	–	3HB	–	Pagliano et al. (2020)
OFSMW	Butyric acid, acetic acid, propionic acid	Mixed cultures	Fed-batch	pH = 8.0–9.0, Temp = 22–25 °C	46	3HB, 3 HV	65 g PHA/kg TVS	Valentino et al. (2019)
Sugarcane molasses	Acetic acid, butyric acid	*Bacillus subtilis*	Batch	pH = 7.0, Temp = 30 °C	70.5	3HD	1.1–2.01 g/L	Rathika et al. (2019)

-: No data, BSG: Brewer's spent grain, OFSMW: Organic fraction of solid municipal waste, Temp: Temperature, 3HB: Poly-3-hydroxybutyrate, 3HD: Poly-3-hexadecanoate, 3HH: Poly-3-hydroxyheptanoate, 3 HV: Poly-3-hydroxyvalerate, 3HB-*co*-3HV: Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate).

The use of these chemical precursors is still in its infancy in PHA studies but some published reports managed to demonstrate their applicability in PHAs. Amulya et al. (2014) used the spent effluents for bioH_2 production and the resulting acidogenic effluents were used as a carbon source for PHA production by *Bacillus tequilensis*. This sequential approach led to an optimal H_2 yield of 39.8 L and a PHA yield of 7.8 g/L. There was a high substrate removal which was associated with the reduction in acidification (53% to 15%). The results demonstrated that the VFAs were effectively utilized by PHA-producers (Amulya et al., 2014).

In another study, Arumugam et al. (2014) reported the co-production of bioH_2 and PHAs from the co-cultures of *Enterobacter aerogenes* and *Rhodobacter sphaeroides* using *Calophyllum inophyllum* oil cake as a carbon source. These co-cultures were strategically applied to convert the effluents into bioH_2 while promoting the accumulation of PHAs as it was revealed that the production of bioH_2 does not compete with PHA production when using biocatalysts. The authors obtained an optimal H_2 and PHA yield of 7.95 L H_2/L media and 10.73 g/L media, respectively, under alternate dark and photo bioprocess conditions (Arumugam et al., 2014).

Meanwhile, Zhao et al. (2019) reported the sequential production of bioH_2 and PHA using acidogenic wastewaters consisting of hydrolyzed-polyacrylamide (HPAM). The HPAM-degrading bacteria was used to synthesize the acid-rich effluents and these were later used for PHA studies by *Bacillus cereus* as the inoculum source. An optimal PHA yield of 54.2% VSS was also attained at an influent (C/N) ratio of 97 (Zhao et al., 2019). Similarly, *Bacillus cereus* was also used in the biosynthesis of bioH_2 and PHAs using acidogenic liquids which composed of Taihu blue algae (Yan et al., 2010). The acidogenic effluents which consisted mainly of acetate and butyrate achieved a maximum PHA yield of 2.26 g/L (Yan et al., 2010).

It was also shown that the acidogenic effluents acquired from industrial wastewaters are suitable for simultaneous production of bioH_2 and biopolymer by various cultures. A two-cascade approach aimed at bioH_2 and PHA production was used to valorize the acidogenic effluents extracted from the cheese industry (Colombo et al., 2019). In this study, the authors applied a sequential process whereby the fermented effluents were first used as carbon sources in the bioH_2 reactors and this led to optimal H_2 yields of 3.47 and 5.07 NL H_2 d^{-1} for cheese whey (CW) and concentrated cheese whey permeate (CCWP), respectively, alongside the organic acids (14.6 and 12.6 g/L. d for CW and CCWP) for both feedstocks. The acid-rich medium from the bioH_2 reactor was then used for PHA studies and this led to a maximum PHA yield of 62 and 55.1 g PHA/g.VSS for CW and CCWP, respectively (Colombo et al., 2019).

Reddy and Mohan (2012) successfully conducted an integrated biorefinery approach involving bioH_2 and PHA production. In this method, the PHA-storing microorganisms used the effluents from the bioH_2 producing reactor as a carbon source. This biorefinery approach led to a high substrate conversion under both anaerobic (72%) and aerobic (78%) microenvironments (Reddy and Mohan, 2012). Likewise, it was demonstrated that the acidogenic process could be integrated into a biorefinery framework whereby multiple products (H_2, CH_4, lipids, and PHAs) could be co-produced leading to zero waste generation (Sitthikitpanya et al., 2021). The bioH_2 effluent produced 321.1 mL/g VS of CH_4 yield, 2.01 g/L PHA concentration, and 0.20 g/L of lipid concentration (Sitthikitpanya et al., 2021). Mohammadi et al. (2012) revealed that the VFAs from the acidogenic fermentation of palm oil mills are beneficial and affordable for PHA production. Whereas Almeida et al. (2021) observed that phototrophic mixed cultures were capable of synthesizing PHAs using VFAs from the acidogenic wastewaters. These reports demonstrate the versatility of the microbial species that can be used for PHA production. Elsewhere, it was reported that the acidogenic effluent extracted from the crude paper industry could be used as a substrate for PHA-storing bacterial species (Tyagi and Sharma, 2021). Therefore, the utilization of industrial effluents in PHA production will be helpful in the alleviation of environmental pollution and will also reduce the high waste disposal costs (Tyagi and Sharma, 2021). Other studies are exploring the use of statistical modelling tools such as Plackett-Burman design and Box-Behnken design towards the optimization of operational variables whereby their individualistic and synergistic effects on PHA yields are evaluated (Sabapathy et al., 2019). This information will be crucial for the advancement of PHA synthesis using biotechnological processes. Halophiles are also regarded as promising cell factories for industrial-scale production of PHAs due to their unique characteristics such as their (i) ability to utilize a wide spectrum of carbon materials including waste-derived VFAs, (ii) high salinity requirements, (iii) ability to function under non-sterile conditions, and (iv) high intracellular osmotic pressure resulting in high PHA recovery (Mitra et al., 2020). Bhattacharyya et al. (2014) attained 71% of PHAs from ethanol stillage consisting of VFAs using *Haloferax mediterranei* which is an extreme halophilic microorganism. Similarly, *Halomonas boliviensis*, another halophic bacterium, used the VFAs from the acidogenic process and produced a high PHA content of 70% (García-Torreiro et al., 2016). This bacterium assimilates various wastes and can convert them into PHAs (Rivera-Terceros et al., 2015).

Owing to its high PHA-accumulating abilities (70–90% DCW) and metabolic versatility, studies are also tailored towards the use of *Cupriavidus necator* for PHA recovery. A more recent study by Jawed et al. (2022) examined the synthesis of PHA using *C. necator* and observed that this inoculum was effective in the utilization of VFAs, resulting in PHA yields of 0.92 to 1.78 g/L. Similarly, Brojanigo et al. (2020) used VFAs derived from starchy wastes for PHA production by *C. necator* DSM 545 through an innovative method known as simultaneous saccharification and fermentation (SSF). In this work, the PHA yields varied from 1.95 to 5.18 g/L when using starchy waste streams (Brojanigo et al., 2020). Fig. 1 shows the production of PHAs using VFAs acquired from different acidogenic effluents.

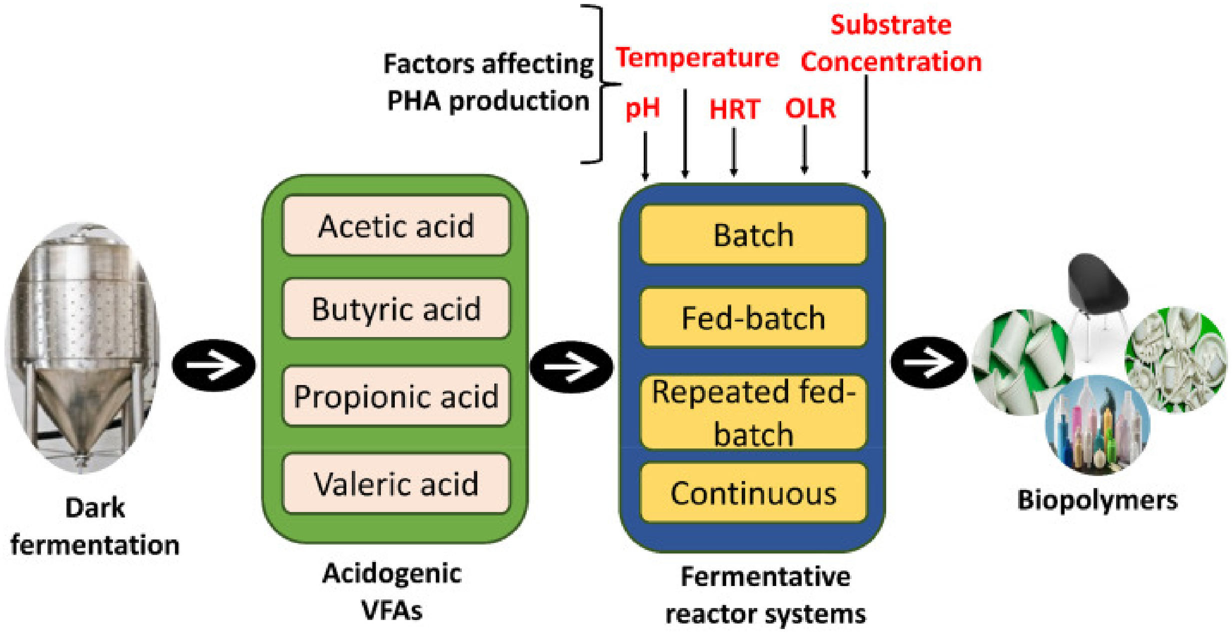


Fig. 1. Schematic representation of PHA production using acidogenic-derived VFAs. Adapted and modified from Banu et al. (2021).

4. Microorganisms used for PHA production

4.1. Pure cultures

PHAs are produced by a broad spectrum of pure cultures with some studies reaching pilot scale (Bellini et al., 2022). Within the microbial cell and under excess carbon, they are stored in the form of intracellular granules under nutrient-limiting conditions of nitrogen, phosphorous, sulphur, or oxygen during fermentation (Verlinden et al., 2007; Serafim et al., 2008). However, nutrient starvation is not required by some PHA-producing strains (Khatami et al., 2021).

To date, >300 microbial species, spanning both bacterial (Gram-positive and Gram-negative) and archaeal domains, have been shown to accumulate PHAs under aerobic and anaerobic conditions (Kim et al., 2007; Khatami et al., 2021). Amongst these species, *Cupriavidus necator* is the most studied bacterium due to its abundance (it is found in soil and water) and can effectively degrade many environmental pollutants such as benzoates, phenols, and *B*-phenyl (Amini et al., 2020). Furthermore, *C. necator* can exhibit heterotrophic and lithoautotrophic pathways depending on its microenvironments (Sohn et al., 2021). As a result of its metabolic traits, various substrates have been used for PHA production and these include agricultural residues (Ng et al., 2010), food waste (Brojanigo et al., 2020), and industrial effluents (Sen et al., 2019). Passanha et al. (2013) showed that this bacterium can produce up to 90% of dry cell weight (DCW) alongside a PHA yield of 12.2 g/L during PHA synthesis.

Scientists have also explored bacteria belonging to the genus *Bacillus* and these can accumulate PHA content varying from 11 to 69% of DCW with PHA yields that range from 3.3 to 70 g/L (Singh et al., 2009; Faccin et al., 2013), with some *Bacillus* sp. capable of producing as much as 90% DCW under nutrient imbalance (Madison and Huisman, 1999). The use of such high PHA-producing species is advantageous because they have a fast growth rate compared to other microbial cultures, can secrete many hydrolytic enzymes, and lack the lipopolysaccharides which make it easier for recovery of PHAs (Khiyami et al., 2011; Mohapatra et al., 2017). *Pseudomonas* sp. are gaining increasing popularity amongst researchers due to their metabolic versatility and their ability to utilize different carbon sources (Poblete-Castro et al., 2014; Mozejko-Ciesielska et al., 2019). These bacterial cultures produced an optimal PHA content of 75.5% DCW and a PHA yield of 1.8 g/L. h when nonanoic acid and glucose were used as co-substrates during a fed-batch cultivation process (Jiang et al., 2013). Other PHA-accumulating strains include *Escherichia coli*, *Burkholderia sacchari*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Enterobacter carcerogenus*, *Alcaligenes latus*, *Azotobacter beijerinckii*, *Brevibacterium halotolerance*, and *Stenotrophomonas rhizoposid*, amongst others (Pungsungvorn and Wisetsing, 2021).

4.1.1. Metabolic engineering

Many studies use pure cultures as they usually produce a high PHA content (70–90%) and these inoculum sources are used to target specific PHA monomer composition (Khatami et al., 2022). However, the reliance on pure cultures presents a lot of constraints in PHA biosynthesis, and these are mainly centered around the: (i) low substrate utilization, (ii) slow microbial growth rates, (iii) need for sterile conditions, and (iv) use of specific sugars. Metabolically-engineered cultures are those microbial species whose genomes have been specifically modified to obtain beneficial phenotypic traits. Herein, the genetic engineering goals focus on addressing some of the abovementioned issues (Rodriguez-Perez et al., 2018; Kumar et al., 2020).

For decades, scientists have used metabolic engineering and classical strain-improvement methods to increase PHAs in microbial cultures to make them commercially applicable. Some of these methods date back to the use of random mutagenesis by subjecting microorganisms to chemicals and UV light (Khatami et al., 2021). For example, a strain of *C. necator* that had undergone chemical mutation was used to increase the fraction of (*R*)-3-hydroxyvalerate in poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P (3HB-co-3 HV)] (Lee et al., 1996).

Adaptive evolution, a classical strain-improvement technique, has been widely used in the enrichment of PHA-producing microbial cultures by several researchers (González-Villanueva et al., 2019; Mohamed et al., 2020; Kusumawardhani et al., 2021).

Metabolic pathways were altered in *E. coli* by over-expressing hydrogenase-3 and acetyl-CoA synthetase to promote the production of hydrogen and PHA. In this manner, the inhibitory metabolites such as acetate and formate were eliminated by driving the metabolic fluxes towards PHA synthesis and this led to high PHA content (99% DCW) (Li et al., 2016). Elsewhere, a sucrose-utilization pathway was constructed in two strains of *Ralstonia eutropha* (NCIMB11599 and 437–540) by introducing the *Mannheimia succiniciproducens* MBEL55E *sacC* gene that encodes β-fructofuranosidase (Park et al., 2015). It was observed that β-fructofuranosidase excreted into the culture medium could hydrolyze sucrose to glucose and fructose, and these sugars were effectively used by the recombinant *R. eutropha* strains, producing high PHA content (73.2% DCW) and PHA yield (1.9 g/L) under N_2 limited conditions (Park et al., 2015). The engineering of *Halomonas* TD01 through CRISPR interference represses the expression of the FtsZ gene, which led to elongated and filamentous bacterial cells that could accumulate higher PHAs than the wild-type control (Tao et al., 2017).

Recently, engineered strains of *Rhodovulum sulfidophilum* increased the PHA content by 1.7-fold, and these strains reached the stationary growth phase after one day of cultivation and accumulated the maximum PHA after two days faster than the wild-type strains (Foong et al., 2021). Therefore, these results are in accordance with literature as it has been reported that an ideal PHA-producer should have a high growth rate so that this will accelerate the consumption of carbon source, which is important when using substrates that are derived from waste streams such as lignocellulosic biomass and VFAs obtained from spent effluents (Ren et al., 2018).

It has also been shown that the engineered strains of *C. necator* can be used in the valorization of lactose-rich wastes and starchy wastes as these residues were previously reported to be ineffective for the growth of *C. necator*. A recent report by Bellini et al. (2022) revealed that these substrates are now applicable in PHA synthesis by recombinant *C. necator*. In the same vein, Brojanigo et al. (2022) demonstrated the use of an engineered amylolytic strain of *C. necator* DSM 545 for PHA synthesis using starchy wastes in a one-step fermentation approach. The recombinant *C. necator* DSM 545 showed promising results with PHA yields of 5.78 and 3.65 g/L for broken rice waste and sweet potato waste, respectively (Brojanigo et al., 2022).

4.2. Mixed cultures

For many years, pure cultures have been the primary focus for bench-scale and pilot-scale production of PHAs as mentioned above (Carvalho et al., 2022). However, the high operational costs that are associated with the use of pure cultures have contributed to a search for other sources of inoculum. Therefore, mixed microbial cultures (MMC) are gaining widespread attention due to their many benefits, such as the ability to use diverse carbon materials, including waste, their ability to operate under non-sterile conditions, their low maintenance costs, and their ability to generate high PHA productivity (Obruča et al., 2022). These microbial cell factories can reduce the overall PHA production costs by >50% compared to pure cultures (Reis et al., 2003). Moreover, MMC are capable of producing PHA with different monomeric units such as hydroxybutyrate (HB) and hydroxyvalerate (HV) (Cruz et al., 2022).

Villano et al. (2014) revealed that MMC could compete with pure cultures in terms of PHA content during a sequential batch process that lasted for four months. The authors observed a PHA content of >90% using a synthetic mixture of acetic and propionic acid (Villano et al., 2014). The mixed culture technology adopts a strategy that mimics the ecological systems of microorganisms where PHA-producing consortia are enriched based on the cultivation conditions that are similar to their native habitats (Silva et al., 2022). Bioprocesses that involve MMC are usually conducted in three sequential steps (Khatami et al., 2021). Firstly, biodegradable compounds such as VFAs are produced using biomass feedstocks through the acidogenic process. Then MMC that have high PHA-storing capabilities are enriched, followed by the accumulation of PHAs in PHA-storing microbial consortia (Khatami et al., 2021). It has been reported that the MMC are subjected to alternating feast and famine regimes (see Fig. 2) in order to boost the proliferation of PHA-accumulating microbial communities (Huang et al., 2018; Ribeiro et al., 2022). Herein, the carbon source is supplied in surplus during the feast regime while it is limited during the famine regime. Moreover, the expression of growth-associated enzymes is suppressed when these organic compounds begin to accumulate and the microbial cultures that can produce PHAs become predominant (Koller et al., 2017). This process is known as culture-enrichment, and it is crucial because the recovery of PHAs is mainly dependent on the performance of these microbial cultures (Serafim et al., 2008). The enrichment process is also dependent on bioprocess parameters such as the type of substrates used, organic loading rate, feast/famine feeding ratio, etc. (Rojas-Z et al., 2021).

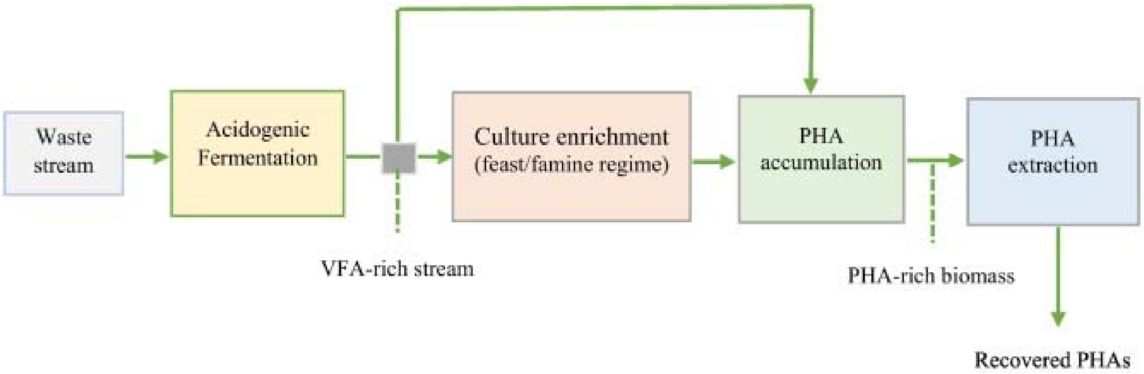


Fig. 2. Schematic illustration of PHA synthesis by MMC. Adapted from Khatami et al. (2021).

A wide variety of organic feedstocks, including organic wastewaters (Bosco and Chiampo, 2010; Tamang et al., 2021; Shen et al., 2022), food waste (Silva et al., 2022), cheese whey (Wang et al., 2022a), and industrial waste (Queirós et al., 2014) have been reported in PHA production using MMC. To ensure that the targeted precursors (VFAs) are successfully extracted, these biomass feedstocks are firstly converted into VFAs using acidogenic bacteria (Fig. 2) so that the unwanted compounds/metabolites do not inhibit MMC during PHA production. Likewise, this is crucial when using acidogenic effluents that have been derived from the DF process as these liquids have a huge composition of VFAs as indicated earlier. Despite numerous studies reporting the biosynthesis of PHAs using MMC obtained from different microenvironments, these cultures have rarely been characterized in the literature.

Therefore, recent efforts are now focusing on the characterization of PHA-storing MMC using molecular techniques. For example, Crognale et al. (2019) studied the diversity of PHA-accumulating bacteria at a pilot scale using a high-throughput 16S rRNA gene sequencing approach. It was revealed that the microbial populations were dominated by *Hydrogenophaga* species, which contributed to high PHA content (Crognale et al., 2019). Another study that examined the impact of pH and C/N ratio on microbial diversity and PHA-accumulation showed that pH 9.0 and nitrogen-starvation were suitable for the bioprocess (Zhou et al., 2022). At pH 9.0, *Thauera* was reported as the most dominant species in all MMC, whereas *Paracoccus* sp. and *Alcaligenes* sp. were the prevalent species at pH 7.0 (Zhou et al., 2022). Other PHA-accumulating microorganisms such as species of *Plasticicunulans*, *Paracoccus*, and *Azoarcus* have also been reported as the dominant species during PHA production via MMC (Wang et al., 2017; Huang et al., 2018; Inoue et al., 2018; Marang et al., 2018).

Thus, having an in-depth understanding of the species that are active in MMC systems will help scientists become more knowledgeable about the cultivation and engineering of these cultures, and this will provide crucial information that will be useful for future scale-up studies.

5. Factors affecting the accumulation of PHAs

5.1. Carbon-nitrogen (C/N) ratio

A study by Sánchez-Valencia et al. (2021) reported that a low C/N ratio enhances the growth of PHA-storing consortia while a high C/N ratio tends to reduce the accumulation of PHAs, probably due to the inhibition that is induced by the high concentration of carbon as these species thrive under nutrient starvation. Herein, the authors evaluated various C/N ratios (13.3 to 42.1) and observed that low C/N ratios of 13.3 and 23.3 favoured PHA synthesis (Sánchez-Valencia et al., 2021). These results are in agreement with other studies that examined the effect of C/N on PHAs (Wang et al., 2007; Kulpreecha et al., 2009; Ahn et al., 2015).

5.2. The types of carbon sources used in PHA synthesis

The carbon source accounts for >50% of the overall costs in PHA production (Urtuvia et al., 2014). Therefore, the use of waste-derived substrates will play a crucial role in the advancement of PHAs due to their affordability as mentioned earlier. Lorini et al. (2021) studied the production of PHAs using different biowastes such as cellulosic primary sludge, organic fraction of municipal solid waste, sewage sludge (OFMSW-WAS), and fruit waste, respectively, from MMC under pilot-scale conditions. OFSMW-WAS was reported as a suitable substrate due to its biodegradability and nutritional composition. Cui et al. (2016) investigated the role of glucose-, starch-, and acetate-rich medium on the enrichment of PHA using MMC. The cultures that were enriched with acetate and glucose produced a PHA content of 64.7% and 60.5% DCW, with corresponding yields of 0.60 and 0.54 mg PHA/mg DCW, respectively. However, the starch-enriched cultures produced a low PHA because starch consists of a complex recalcitrant structure that must undergo vigorous pretreatment before being used in PHAs (Cui et al., 2016). This work was also comparable to other PHA studies that used complex substrates such as molasses, whey, cellulose, hemicellulose, and palm oil, where the PHA content was lower than that of simple sugars (Castilho et al., 2009).

More recently, Lagoa-Costa et al. (2022) produced PHAs by co-digesting brewery waste and cheese whey at different ratios. By increasing the feedstock mix-ratio from 0 to 50%, these carbon materials did not only alter the PHA content but also the PHA-accumulating cultures i.e., bacteria such as *Bifidobacterium*, *Caproiciproducens*, and *Megasphaera* were the most dominant species during the early stages of the co-digestion. Whereas new microbial assemblages emerged after the mix-ratio of 25% which led to the proliferation of species like *Ethanoligenens* and *Selenomonas* (Lagoa-Costa et al., 2022). In another similar study, Carvalho et al. (2018) evaluated the impact of alternating the feedstocks on PHA production using mixed cultures. It was observed that changing the feedstocks also altered the microbial community profile. *Firmicutes* was the most dominant phylum when cheese whey was used as the carbon source. Whereas *Actinobacteria* were the dominant species when sugarcane molasses was used as a carbon source. These results, therefore, underscore the importance of choosing a suitable carbon source as it also affects the diversity of microbial species in MMC.

Lemos et al. (1998) studied how various types of VFAs such as acetate, butyrate, and propionate affected the synthesis of biopolymers. Herein, acetate led to a high composition of hydroxybutyrate (HB) (75.25%), while butyrate and propionate only produced 59.68% and 28.6% of HV, respectively (Lemos et al., 1998). These findings could be instrumental in the selection of the most suitable VFA-type, particularly when using VFAs from waste streams.

5.3. Medium pH

pH is an important parameter as it impacts the overall metabolism of PHA-storing species (Li et al., 2020). A perusal of studies focusing on the effect of pH on PHA biosynthesis showed that a broad pH range (5.0–9.0) is applicable in this process, particularly when dealing with microbial cultures that are considered to be non-extremophiles (Villano et al., 2010; Li et al., 2020), although higher pH values (7.0–9.0) have been shown to yield optimal PHA content in comparison to low pH values (Chua et al., 2003; Chen et al., 2013).

Villano et al. (2010) observed that increasing the pH (6.0 to 9.5) increased the hydroxyvalerate (HV) content in the copolymer by around 48% during PHA production using MMC. The authors also concluded that controlling pH could be beneficial when targeting certain types of polymers such as HV (Villano et al., 2010). Contradicting results have also been reported in the literature. For instance, Serafim et al. (2004) showed that uncontrolled pH favoured the production of PHAs as 12% of substrate contributed towards energy generation, while 88% was used for microbial growth and PHA accumulation. Another similar study that synthesized PHAs using fermented-molasses revealed that members of the genera *Thauera*, *Paracoccus,* and *Azoarcus* were the most dominant species during PHA production, resulting in a PHA content of up to 60% and a PHA yield of 0.68 C-mol PHA/C-mol VFA (Albuquerque et al., 2013). Moreover, Montiel-Jarillo et al. (2017) observed a high PHA-storing capacity in MMC that were cultured without pH control under N_2 limited conditions. This phenomenon may be caused by the fact that the microbial cultures used in these studies are tolerant to diverse growth conditions. This implies that more research is needed to explain the role of pH in PHA-storing microbial cultures and this information will be useful for future large-scale studies. Meanwhile, lower pH (5.0 to 5.5) values have been shown to benefit the first step of PHA production (acidogenesis) where substrates are converted into VFA-rich metabolites, which are later used by PHA-accumulators (Albuquerque et al., 2007; Bengtsson et al., 2008a).

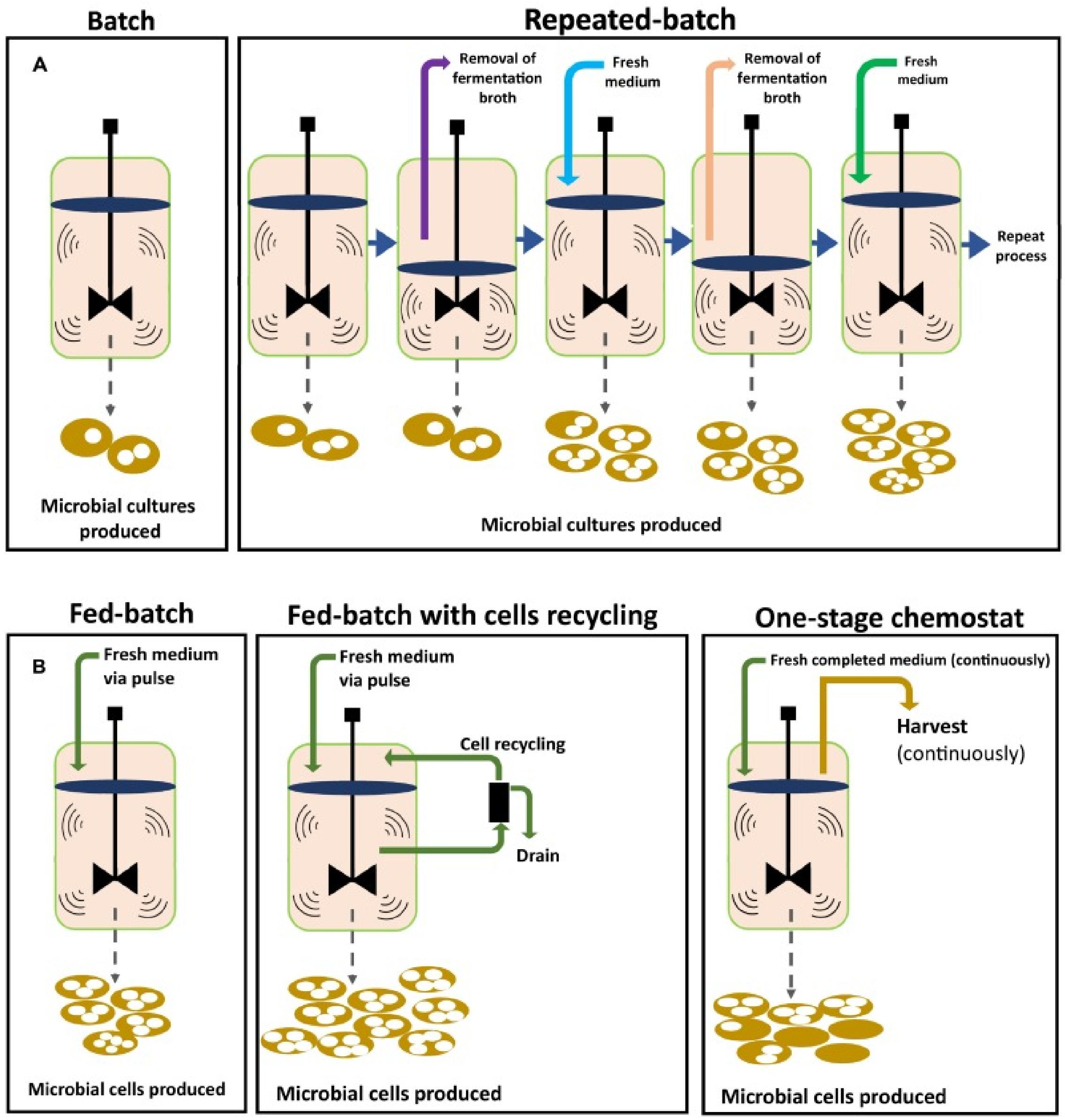
5.4. Fermentative reactor systems

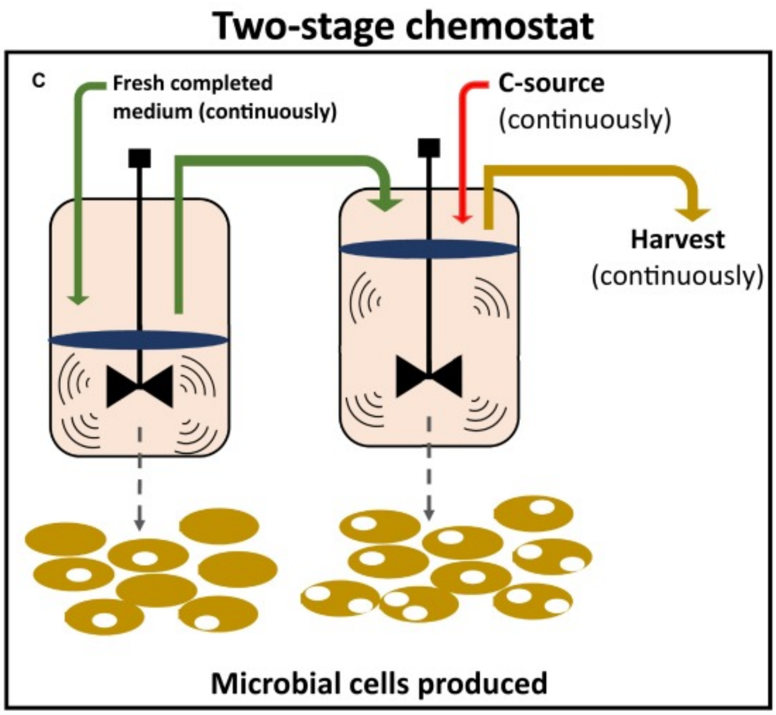
Different types of reactor systems have been implemented in PHA production. These include batch reactors, fed-batch reactors, and continuous stirred tank reactors (McAdam et al., 2020). Batch reactors are common in PHAs because of their simplicity and cost-effectiveness.

However, other types of reactor systems are being explored in PHA biosynthesis due to the fact that batch reactors generate low productivity. Hence, recent studies are now producing PHAs via fed-batch and continuous reactors. Fed-batch systems are favoured because they enhance the accumulation of PHA-producers as they lead to high cell densities and this is accomplished by ensuring that there is a constant supply of nutrients within the reactors (Blunt et al., 2018).

Singh et al. (2021) used a cyclic fed-batch system for PHA production and this fermentation strategy generated a PHA yield that was four times higher than that of a batch process. Likewise, Jiang et al. (2013) used the fed-batch process to generate an optimum PHA content of 75.5% and a PHA yield of 1.8 g/L. h using glucose and nonanoic acid as co-substrates. Other studies that used low-cost carbon sources such as agro-waste or animal waste revealed that fed-batch processes are also well-suited for the synthesis of PHAs (Kulpreecha et al., 2009; Cesário et al., 2014; Mozejko and Ciesielski, 2014; Wang et al., 2022b).

Although batch and fed-batch systems are established methods of producing PHAs, ongoing research evaluates the use of continuous bioprocesses in PHA studies due to their ability to (i) operate under steady-state, (iii) sustain constant pH and nutrients supply, (iii) maximize the concentration of the product, and (iv) its suitability for large-up bioprocess. Nonetheless, it is impossible to produce PHAs using a one-step continuous fermentation system since PHAs are synthesized under nutrient-limited conditions i.e., the microorganisms are fed with the essential nutrients to initiate biomass growth, thereafter the nutrients are depleted to trigger the biosynthesis of PHAs (McAdam et al., 2020). Therefore, researchers are exploring two-stage and multi-stage continuous fermenters in an attempt to overcome this limitation (Sharma, 2019; Heepkaew and Suwannasilp, 2020). Jung et al. (2001) successfully developed a two-step continuous bioprocess which led to >60% of PHA. Chmelová et al. (2022) also produced a biomass yield that was 1.7-higher than the conventional medium using *Pseudomonas oleovorans* as an inoculum source. Nevertheless, the use of continuous bioprocesses in PHAs is still restricted by the high operating costs in comparison to the well-known modes (batch and fed-batch fermentations). In addition, the aforementioned reactor systems are also dependent on bioprocess conditions such as pH, temperature, dissolved oxygen, aeration rate, impeller design, agitation speed, etc. (Surendran et al., 2020). Fig. 3 provides a schematic representation of the reactor systems that are used in PHA production.


Batch
A
Microbial cultures produced
Repeated-batch
Removal of fermentation broth
Fresh medium
Removal of fermentation broth
Fresh medium
Repeat process
Microbial cultures produced
Fed-batch
B
Fresh medium via pulse
Microbial cells produced
Fed-batch with cells recycling
Fresh medium via pulse
Cell recycling
Drain
Microbial cells produced
One-stage chemostat
Fresh completed medium (continuously)
Harvest (continuously)
Microbial cells produced



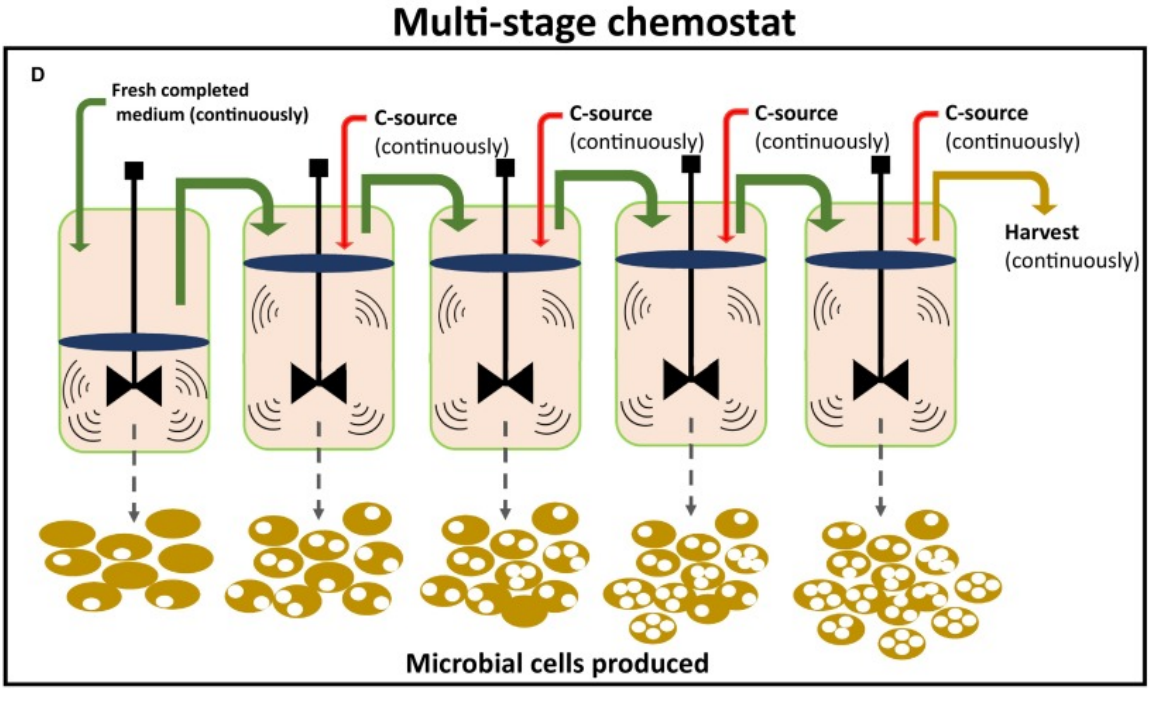


Fig. 3. The reactor systems (A-D) used in the synthesis of PHAs. Adapted and modified from McAdam et al. (2020).

6. Current challenges and suggestions for future studies

The industrialization of PHAs might offer a lot of benefits to the polymer industry, especially when it comes to the mitigation of environmental pollution and manufacturing costs as documented in this review. However, a lot of hurdles need to be overcome before this biotechnological process can compete with petroleum-based processes. Most PHA-producing processes are carried out at bench-scale conditions which imply that the process dynamics for large-scale PHA production are not well understood. Hence, this implies that more studies should be conducted at large-scale to acquire deeper insights into the operational conditions that affect the overall performance of PHA-accumulating consortia. The few pilot-scale studies that are available in the literature rely mostly on genetically-engineered strains and these typically require stringent bioprocess conditions (specific carbon source, high-level of sterilization, regulated fermentation conditions, etc). This challenge could be circumvented by the use of halophilic consortia as these species can thrive under diverse growth conditions and can assimilate various carbon sources including wastes (Mitra et al., 2020). They are also preferred because they can be cultivated under non-sterile fermentation conditions and holds a huge potential for large-scale studies in PHAs (Thomas et al., 2020).

Other pressing issues in PHAs include the low substrate conversion and accumulation of unwanted by-products during PHA synthesis which ultimately reduces the PHA yields. Researchers have suggested the use of metabolic tools such as genetic engineering to avert these issues as the metabolic fluxes are directed towards PHA synthesis (Zhang et al., 2020; Guleria et al., 2022). However, the cost implications will need to be studied to see if such tools are economically-feasible in PHAs. The use of MMC also presents its challenges in PHA synthesis as some microbial assemblages compete with the dominant PHA-producers during the upstream process, resulting in low PHA yields (Yadav et al., 2020). Bioaugmentation strategies focusing on the predominant PHA-accumulators will therefore be instrumental in the enrichment of PHA yields during the feast-famine regimes (Khatami et al., 2021).

The recovery of PHAs comes with its problems as well because solvents and other chemicals are used to recover/extract the PHAs from microbial cells and this is expensive. Additional costs are also incurred as these chemicals need to be safely disposed into the environment (Wang et al., 2014). It is therefore imperative for scientists to search for inexpensive extraction techniques and chemicals that are environmentally-friendly during downstream processing.

7. Conclusion

It is therefore evident from this review that PHAs could play a crucial role in the bio-based industry. However, the industrialization of PHAs is still hindered by the high operational costs because most scale-up studies rely mostly on recombinant strains which require stringent bioprocess conditions coupled with the costly downstream process. Research is ongoing to search for inexpensive and scalable PHA processes. This includes the use of cheap substrates and inoculum sources that are ideal for large-scale, the incorporation of mathematical models to optimize the process conditions, and designing metabolic tools that can obtain high yields.

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.

References

Ahn, J., Jho, E.H., Nam, K., 2015. Effect of C/N ratio on polyhydroxyalkanoates (PHA) accumulation by Cupriavidus necator and its implication on the use of rice straw hydrolysates. Environ. Eng. Res. 20, 246–253. https://doi.org/10.4491/eer.2015.055.

Albuquerque, M.G.E., Eiroa, M., Torres, C., Nunes, B.R., Reis, M.A.M., 2007. Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. J. Biotechnol. 130, 411–421. https://doi.org/10.1016/j.jbiotec.2007.05.011.

Albuquerque, M.G.E., Carvalho, G., Kragelund, C., Silva, A.F., Crespo, B.M.T., Reis, M.A. M., 2013. Link between microbial composition and carbon substrate-uptake preferences in a PHA-storing community. ISME J. 7, 1–12. https://doi.org/10.1038/ismej.2012.74.

Almeida, J.R., Serrano, E., Fernandez, M., Fradinho, J.C., Oehmen, A., Reis, M.A.M., 2021. Polyhydroxyalkanoates production from fermented domestic wastewater using phototrophic mixed cultures. Water Res. 197, 117101 https://doi.org/10.1016/j.watres.2021.117101.

Amini, M., Yousefi-Massumabad, H., Younesi, H., Abyar, H., Bahramifar, N., 2020. Production of the polyhydroxyalkanoate biopolymer by Cupriavidus necator using beer brewery wastewater containing maltose as a primary carbon source. J. Environ. Chem. Eng. 8, 103588 https://doi.org/10.1016/j.jece.2019.103588.

Amulya, K., Reddy, V.M., Venkata Mohan, S., 2014. Acidogenic spent wash valorization through polyhydroxyalkanoate (PHA) synthesis coupled with fermentative biohydrogen production. Bioresour. Technol. 158, 336–342. https://doi.org/10.1016/J.BIORTECH.2014.02.026.

Aremu, M.O., Ishola, M.M., Taherzadeh, M.J., 2021. Polyhydroxyalkanoates (PHAs) production from Volatile Fatty Acids (VFAs) from Organic Wastes by Pseudomonas oleovorans. Fermentation 7, 287. https://doi.org/10.3390/FERMENTATION7040287.

Arumugam, A., Sandhya, M., Ponnusami, V., 2014. Biohydrogen and polyhydroxyalkanoate co-production by Enterobacter aerogenes and Rhodobacter sphaeroides from Calophyllum inophyllum oil cake. Bioresour. Technol. 164, 170–176. https://doi.org/10.1016/j.biortech.2014.04.104.

Banu, R.J., Ginni, G., Kavitha, S., Kannah, Y.R., Kumar, V., Kumar, A.S., Gunasekaran, M., Tyagi, V.K., Kumar, G., 2021. Polyhydroxyalkanoates synthesis using acidogenic fermentative effluents. Int. J. Biol. Macromol. 193, 2079–2092. https://doi.org/10.1016/J.IJBIOMAC.2021.11.040.

Baumann, I., Westermann, P., 2016. Microbial production of short chain fatty acids from lignocellulosic biomass: current processes and market. Biomed. Res. Int. 2016 https://doi.org/10.1155/2016/8469357.

BCC Research, 2019. Oleochemical Fatty Acids: Global Markets to 2023. Available at.

Bellini, S., Tommasi, T., Fino, D., 2022. Poly(3-hydroxybutyrate) biosynthesis by Cupriavidus necator: a review on waste substrates utilization for a circular economy approach. Bioresour. Technol. Rep. 17, 100985 https://doi.org/10.1016/j.biteb.2022.100985.

Bengtsson, S., Hallquist, J., Werker, A., Welander, T., 2008a. Acidogenic fermentation of industrial wastewaters: effects of chemostat retention time and pH on volatile fatty acids production. Biochem. Eng. J. 40, 492–499. https://doi.org/10.1016/j.bej.2008.02.004.

Bengtsson, S., Werker, A., Christensson, M., Welander, T., 2008b. Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. Bioresour. Technol. 99, 509–516. https://doi.org/10.1016/J.BIORTECH.2007.01.020.

Bhattacharyya, A., Saha, J., Haldar, S., Bhowmic, A., Mukhopadhyay, U.K., Mukherjee, J., 2014. Production of poly-3-(hydroxybutyrate-co-hydroxyvalerate) by Haloferax mediterranei using rice-based ethanol stillage with simultaneous recovery and re-use of medium salts. Extremophiles 18, 463–470. https://doi.org/10.1007/s00792-013-0622-9.

Blunt, W., Levin, D.B., Cicek, N., 2018. Bioreactor operating strategies for improved polyhydroxyalkanoate (PHA) productivity. Polymers 10. https://doi.org/10.3390/polym10111197.

Bosco, F., Chiampo, F., 2010. Production of polyhydroxyalcanoates (PHAs) using milk whey and dairy wastewater activated sludge production of bioplastics using dairy residues. J. Biosci. Bioeng. 109, 418–421. https://doi.org/10.1016/J.JBIOSC.2009.10.012.

Brojanigo, S., Parro, E., Cazzorla, T., Favaro, L., Basaglia, M., Casella, S., 2020. Conversion of Starchy Waste Streams into Polyhydroxyalkanoates using Cupriavidus necator DSM 545. Polymers 12, 1496. https://doi.org/10.3390/polym12071496.

Brojanigo, S., Gronchi, N., Cazzorla, T., Wong, T.S., Basaglia, M., Favaro, L., Casella, S., 2022. Engineering Cupriavidus necator DSM 545 for the one-step conversion of starchy waste into polyhydroxyalkanoates. Bioresour. Technol. 347, 126383 https://doi.org/10.1016/j.biortech.2021.126383.

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. New Biotechnol. 40, 207–217. https://doi.org/10.1016/j.nbt.2017.08.007.

Carvalho, J.M., Marreiros, B.C., Reis, M.A.M., 2022. Polyhydroxyalkanoates production by mixed microbial culture under high salinity. Sustain. 14, 1346. https://doi.org/10.3390/su14031346.

Castilho, L.R., Mitchell, D.A., Freire, D.M.G., 2009. Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. Bioresour. Technol. 100, 5996–6009. https://doi.org/10.1016/j.biortech.2009.03.088.

Cesário, M.T., Raposo, R.S., de Almeida, M.C.M.D., van Keulen, F., Ferreira, B.S., da Fonseca, M.M.R., 2014. Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. New Biotechnol. 31, 104–113.

https://doi.org/10.1016/j.nbt.2013.10.004.

Chen, H., Meng, H., Nie, Z., Zhang, M., 2013. Polyhydroxyalkanoate production from fermented volatile fatty acids: effect of pH and feeding regimes. Bioresour. Technol. 128, 533–538. https://doi.org/10.1016/j.biortech.2012.10.121.

Chmelová, D., Legerská, B., Ondrejovič, M., Miertuš, S., 2022. Optimization of propagation medium for enhanced polyhydroxyalkanoate production by Pseudomonas oleovorans. Fermentation 8, 16. https://doi.org/10.3390/fermentation8010016.

Chua, A.S.M., Takabatake, H., Satoh, H., Mino, T., 2003. Production of polyhydroxy-alkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent. Water Res. 37, 3602–3611. https://doi.org/10.1016/S0043-1354(03)00252-5.

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. 95, 22–31. https://doi.org/10.1016/j.wasman.2019.05.052.

Crognale, S., Tonanzi, B., Valentino, F., Majone, M., Rossetti, S., 2019. Microbiome dynamics and phaC synthase genes selected in a pilot plant producing polyhydroxyalkanoate from the organic fraction of urban waste. Sci. Total Environ. 689, 765–773. https://doi.org/10.1016/j.scitotenv.2019.06.491.

Cruz, R.A.P., Oehmen, A., Reis, M.A.M., 2022. The impact of biomass withdrawal strategy on the biomass selection and polyhydroxyalkanoates accumulation of mixed microbial cultures. New Biotechnol. 66, 8–15. https://doi.org/10.1016/j.nbt.2021.08.004.

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. 6 https://doi.org/10.1038/srep30766.

Domingos, J.M.B., Puccio, S., Martinez, G.A., Amaral, N., Reis, M.A.M., Bandini, S., Fava, B., 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. 336, 47–53. https://doi.org/10.1016/J.CEJ.2017.11.024.

Dwidar, M., Park, J.Y., Mitchell, R.J., Sang, B.I., 2012. The future of butyric acid in industry. Sci. World J. 2012, 471417 https://doi.org/10.1100/2012/471417.

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. 48, 420–425. In: https://doi.10.1016/j.procbio.2013.02.004.

Foong, C.P., Higuchi-Takeuchi, M., Ohtawa, K., Asai, T., Liu, H., Ozeki, Y., Numata, K., 2021. Engineered mutants of a marine photosynthetic purple nonsulfur bacterium with increased volumetric productivity of polyhydroxyalkanoate bioplastics. ACS Synth. Biol. 11, 909–920. https://doi.org/10.1021/acssynbio.1c00537.

García-Torreiro, M., Lú-Chau, T.A., Steinbüchel, A., Lemaa, J.M., 2016. Waste to bioplastic conversion by the moderate halophilic bacterium Halomonas boliviensis. Chem. Eng. Trans. 49, 163–168. https://doi.org/10.3303/CET1649028.

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 https://doi.org/10.3390/ijms20225737.

Guleria, S., Singh, H., Sharma, V., Bhardwaj, N., Arya, S.K., Puri, S., Khatri, M., 2022. Polyhydroxyalkanoates production from domestic waste feedstock: a sustainable approach towards bio-economy. J. Clean. Prod. 340, 130661 https://doi.org/10.1016/J.JCLEPRO.2022.130661.

Heepkaew, P., Suwannasilp, B.B., 2020. Polyhydroxyalkanoate production using two-stage continuous stirred tank activated sludge systems with glycerol as a carbon source. J. Chem. Technol. Biotechnol. 95, 1180–1190. https://doi.org/10.1002/jctb.6304.

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. 131, 167–176. https://doi.org/10.1016/j.watres.2017.12.033.

Hughes, S.R., Jones, M.A., 2020. The global demand for biofuels and biotechnology-derived commodity chemicals. In: Vertès, A.A., Qureshi, N., Blaschek, H.P., Yukawa, H. (Eds.), Green Energy to Sustainability: Strategies for Global Industries. John Wiley & Sons, New York, pp. 137–156.

Iglesias-Iglesias, R., Portela-Grandío, A., Treu, L., Campanaro, S., Kennes, C., Veiga, M.C., 2021. Co-digestion of cheese whey with sewage sludge for caproic acid production: role of microbiome and polyhydroxyalkanoates potential production. Bioresour. Technol. 337 https://doi.org/10.1016/j.biortech.2021.125388.

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.

Jawed, K., Irorere, V.U., Bommareddy, R.R., Minton, N.P., Kovács, K., 2022. Establishing mixotrophic growth of Cupriavidus necator H16 on CO2 and volatile fatty acids. Fermentation 8, 125. https://doi.org/10.3390/fermentation8030125.

Jayakrishnan, U., Deka, D., Das, G., 2021. Waste as feedstock for polyhydroxyalkanoate production from activated sludge: Implications of aerobic dynamic feeding and acidogenic fermentation. J. Environ. Chem. Eng. 9, 105550 https://doi.org/10.1016/j.jece.2021.105550.

Jiang, X.J., Sun, Z., Ramsay, J.A., Ramsay, B.A., 2013. Fed-batch production of MCL-PHA with elevated 3-hydroxynonanoate content. AMB Express 3, 50. https://doi.org/10.1186/2191-0855-3-50.

Jung, K., Hazenberg, W., Prieto, M., Witholt, B., 2001. Two-stage continuous process development for the production of medium-chain-length poly(3-hydroxyalkanoates). Biotechnol. Bioeng. 72, 19–24. https://doi.org/10.1002/1097-0290(20010105)72: 1<19::AID-BIT3>3.0.CO;2-B.

Kalia, V.C., Singh Patel, S.K., Shanmugam, R., Lee, J.K., 2021. Polyhydroxyalkanoates: Trends and advances toward biotechnological applications. Bioresour. Technol. 326, 124737 https://doi.org/10.1016/J.BIORTECH.2021.124737.

Khatami, K., Perez-Zabaleta, M., Owusu-Agyeman, I., Cetecioglu, Z., 2021. Waste to bioplastics: how close are we to sustainable polyhydroxyalkanoates production? Waste Manag. 119, 374–388. https://doi.org/10.1016/j.wasman.2020.10.008.

Khatami, K., Perez-Zabaleta, M., Cetecioglu, Z., 2022. Pure cultures for synthetic culture development: next level municipal waste treatment for polyhydroxyalkanoates production. J. Environ. Manag. 305, 114337 https://doi.org/10.1016/j.jenvman.2021.114337.

Khiyami, M.A., Al-fadual, S.M., Bahklia, A.H., 2011. Polyhydroxyalkanoates production via bacillus PCS biofilm and date palm syrup. J. Med. Plants Res. 5, 3312–3320.

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., Maršálek, L., de Sousa Dias, M.M., Braunegg, G., 2017. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. New Biotechnol. 37, 24–38. https://doi.org/10.1016/j.nbt.2016.05.001.

Kulpreecha, S., Boonruangthavorn, A., Meksiriporn, B., Thongchul, N., 2009. Inexpensive fed-batch cultivation for high poly(3-hydroxybutyrate) production by a new isolate of bacillus megaterium. J. Biosci. Bioeng. 107, 240–245. https://doi.org/10.1016/j.jbiosc.2008.10.006.

Kumar, G., Ponnusamy, V.K., Bhosale, R.R., Shobana, S., Yoon, J.-J., Bhatia, S.K., Banu, R.J., Kim, S.-H., 2019. A review on the conversion of volatile fatty acids to polyhydroxy-alkonates using dark fermentative effluents from hydrogen production. Bioresour. Technol. 287, 121427 https://doi.org/10.1016/j.biortech.2019.121427.

Kumar, M., Rathour, R., Singh, R., Sun, Y., Pandey, A., Gnansounou, E., Lin, K.-Y.A., Thakur, I.S., Tsang, D.C.W., 2020. Bacterial polyhydroxyalkanoates: Opportunities, challenges, and prospects. J. Clean. Prod. 263, 121500. https://doi.org/10.1016/j.jclepro.2020.121500.

Kusumawardhani, H., Furtwängler, B., Blommestijn, M., Kaltenytė, A., van der Poel, J., Kolk, J., Hosseini, R., de Winde, J.H., 2021. Adaptive laboratory evolution restores solvent tolerance in plasmid-cured Pseudomonas putida S12: a molecular analysis. Appl. Environ. Microbiol. 87, 1–18. https://doi.org/10.1128/AEM.00041-21.

Lagoa-Costa, B., Kennes, C., Veiga, M.C., 2022. Influence of feedstock mix ratio on microbial dynamics during acidogenic fermentation for polyhydroxyalkanoates production. J. Environ. Manag. 303, 114132 https://doi.org/10.1016/J.JENVMAN.2021.114132.

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. 81, 255–258. https://doi.org/10.1016/0922-338X(96)82217-3.

Lemos, P.C., Viana, C., Salgueiro, E.N., Ramos, A.M., Crespo, J.P.S.G., Reis, M.A.M., 1998. Effect of carbon source on the formation of polyhydroxyalkanoates (PHA) by a phosphate-accumulating mixed culture. Enzym. Microb. Technol. 22, 662–671. https://doi.org/10.1016/S0141-0229(97)00243-3.

Li, T., Ye, J., Shen, R., Zong, Y., Zhao, X., Lou, C., Chen, G.-Q., 2016. Semirational approach for ultrahigh poly(3-hydroxybutyrate) Accumulation in Escherichia coli by combining one-step library construction and high-throughput screening. ACS Synth. Biol. 5, 1308–1317. https://doi.org/10.1021/acssynbio.6b00083.

Li, D., Yin, F., Ma, X., 2020. Towards biodegradable polyhydroxyalkanoate production from wood waste: using volatile fatty acids as conversion medium. Bioresour. Technol. 299 https://doi.org/10.1016/j.biortech.2019.122629.

Li, X., Sui, K., Zhang, J., Liu, X., Xu, Q., Wang, D., Yang, Q., 2022. Revealing the Mechanisms of Rhamnolipid Enhanced Hydrogen Production From Dark Fermentation of Waste Activated Sludge, 806. https://doi.org/10.1016/j.scitotenv.2021.150347.

Lorini, L., Martinelli, A., Capuani, G., Frison, N., Reis, M., Sommer Ferreira, B., Villano, M., Majone, M., Valentino, F., 2021. Characterization of polyhydroxyalkanoates produced at pilot scale from different organic wastes. Front. Bioeng. Biotechnol. 9, 52. https://doi.org/10.3389/fbioe.2021.628719.

Madison, L.L., Huisman, G.W., 1999. Metabolic Engineering of Poly(3-Hydroxyalkanoates): from DNA to Plastic. Microbiol. Mol. Biol. Rev. 63, 21–53. https://doi.org/10.1128/mmbr.63.1.21-53.1999.

Marang, L., van Loosdrecht, M.C.M., Kleerebezem, R., 2018. Enrichment of PHA-producing bacteria under continuous substrate supply. New Biotechnol. 41, 55–61. https://doi.org/10.1016/j.nbt.2017.12.001.

Martinez, G.A., Rebecchi, S., Decorti, D., Domingos, J.M.B., Natolino, A., Del Rio, D., Bertin, L., Da Porto, C., Fava, F., 2016. Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. Green Chem. 18, 261–270. https://doi.org/10.1039/C5GC01558H.

Martínez-Avila, O., Llenas, L., Ponsá, S., 2022. Sustainable polyhydroxyalkanoates production via solid-state fermentation: influence of the operational parameters and scaling up of the process. Food Bioprod. Proc. 132, 13–22. https://doi.org/10.1016/J.FBP.2021.12.002.

McAdam, B., Fournet, M.B., McDonald, P., Mojicevic, M., 2020. Production of polyhydroxybutyrate (PHB) and factors impacting its chemical and mechanical characteristics. Polymers 12, 1–20. https://doi.og/10.3390/polym12122908.

Mhlongo, S.I., Ezeokoli, O.T., Roopnarain, A., Ndaba, B., Sekoai, P.T., Habimana, O., Pohl, C.H., 2021. The potential of Single-Cell oils Derived from Filamentous Fungi as Alternative Feedstock sources for biodiesel Production Diversity of Oleaginous Filamentous. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.637381.

Mitra, R., Xu, T., Xiang, H., Han, J., 2020. Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. Microb. Cell Factories 19, 1–30. https://doi.org/10.1186/S12934-020-01342-Z.

Mohamed, E.T., Werner, A.Z., Salvachúa, D., Singer, C.A., Szostkiewicz, K., Rafael Jiméne-Díaz, M., et al., 2020. Adaptive laboratory evolution of Pseudomonas putida KT2440 improves p-coumaric and ferulic acid catabolism and tolerance. Metab. Eng. Commun. 11, e00143 https://doi.org/10.1016/j.mec.2020.e00143.

Mohammadi, M., Hassan, M.A., Shirai, Y., Man, H.C., Ariffin, H., Yee, L.N., Mumtaz, T., Chong, M.-L., Phang, L.-Y., 2012. Separation and purification of polyhydroxyalkanoates from newly isolated Comamonas sp. EB172 by simple digestion with sodium hydroxide. Sep. Sci. Technol. 47, 534–541. https://doi.org/10.1080/01496395.2011.615788.

Mohapatra, S., Maity, S., Dash, H.R., Das, S., Pattnaik, S., Rath, C.C., Samantaray, D., 2017. Bacillus and biopolymer: prospects and challenges. Biochem. Biophys. Rep. 12, 206–213. https://doi.org/10.1016/j.bbrep.2017.10.001.

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.

Mozejko, J., Ciesielski, S., 2014. Pulsed feeding strategy is more favorable to medium-chain-length polyhydroxyalkanoates production from waste rapeseed oil. Biotechnol. Prog. 30, 1243–1246. https://doi.org/10.1002/btpr.1914.

Mozejko-Ciesielska, J., Szacherska, K., Marciniak, P., 2019. Pseudomonas species as producers of eco-friendly polyhydroxyalkanoates. J. Polym. Environ. 27, 1151–1166. https://doi.org/10.1007/s10924-019-01422-1.

Ng, K.-S., Ooi, W.-Y., Goh, L.-K., Shenbagarathai, R., Sudesh, K., 2010. Evaluation of jatropha oil to produce poly(3-hydroxybutyrate) by Cupriavidus necator H16. Polym.Degrad. Stabil. 95, 1365–1369. https://doi.org/10.1016/j.polymdegradstab.2010.01.021.

Novelli, D.L., Moreno Sayavedra, S., Rene, E.R., 2021. Polyhydroxyalkanoate (PHA) production via resource recovery from industrial waste streams: a review of techniques and perspectives. Bioresour. Technol. 331, 124985 https://doi.org/10.1016/j.biortech.2021.124985.

Obruča, S., Dvořák, P., Sedláček, P., Koller, M., Sedlář, K., Pernicová, I., Šafránek, D., 2022. Polyhydroxyalkanoates synthesis by halophiles and thermophiles: towards sustainable production of microbial bioplastics. Biotechnol. Adv. 58, 107906 https://doi.org/10.1016/j.biotechadv.2022.107906.

Pagliano, G., Gugliucci, W., Torrieri, E., Piccolo, A., Cangemi, S., Di Giuseppe, F.A., Robertiello, A., Faraco, V., Pepe, O., Ventorino, V., 2020. Polyhydroxyalkanoates (PHAs) from dairy wastewater effluent: bacterial accumulation, structural characterization and physical properties. Chem. Biol. Technol. Agric. 7, 1–14. https://doi.org/10.1186/S40538-020-00197-1/TABLES/2.

Pal, P., Nayak, J., 2017. Acetic acid production and purification: critical review towards process intensification. Sep. Pur. Rev. 46, 44–61. https://doi.org/10.1080/15422119.2016.1185017.

Pandey, A.K., Pilli, S., Bhunia, P., Tyagi, R.D., Surampalli, R.Y., Zhang, T.C., Kim, S.-H., Pandey, A., 2022. Dark fermentation: production and utilization of volatile fatty acid from different wastes- a review. Chemosphere 288, 132444. https://doi.org/10.1016/j.chemosphere.2021.132444.

Park, S.J., Jang, Y.A., Noh, W., Oh, Y.H., Lee, H., David, Y., et al., 2015. Metabolic engineering of Ralstonia eutropha for the production of polyhydroxyalkanoates from sucrose. Biotechnol. Bioeng. 112, 638–643. https://doi.org/10.1002/bit.25469.

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. 147, 345–352. https://doi.org/10.1016/j.biortech.2013.08.050.

Poblete-Castro, I., Rodriguez, A.L., Chi Lam, C.M., Kessler, W., 2014. Improved production of medium-chain-length polyhydroxyalkanoates in glucose-based fed-batch cultivations of metabolically engineered Pseudomonas putida strains. J. Microbiol. Biotechnol. 24, 59–69. https://doi.org/10.4014/jmb.1308.08052.

Pungsungvorn, N., Wisetsing, A., 2021. Short Communication: Isolation and screening of polyhydroxylalkanoates producing microorganisms from Thailand. Biodiversitas J. Biol. Divers. 22 https://doi.org/10.13057/biodiv/d221131.

Queirós, D., Rossetti, S., Serafim, L.S., 2014. PHA production by mixed cultures: a way to valorize wastes from pulp industry. Bioresour. Technol. 157, 197–205. https://doi.org/10.1016/j.biortech.2014.01.099.

Rajendran, N., Han, J., 2022. Techno-economic analysis of food waste valorization for integrated production of polyhydroxyalkanoates and biofuels. Bioresour. Technol. 348, 126796 https://doi.org/10.1016/j.biortech.2022.126796.

Rathika, R., Janaki, V., Shanthi, K., Kamala-Kannan, S., 2019. Bioconversion of agro-industrial effluents for polyhydroxyalkanoates production using Bacillus subtilis RS1. Int. J. Environ. Sci. Technol. 16, 5725–5734. https://doi.org/10.1007/s13762-018-2155-3.

Raza, Z.A., Abid, S., Banat, I.M., 2018. Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. Int. Biodeterior. Biodegrad. 126, 45–56. https://doi.org/10.1016/j.ibiod.2017.10.001.

Reddy, M.V., Mohan, S.V., 2012. Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. Bioresour. Technol. 103, 313–321. https://doi.org/10.1016/j.biortech.2011.09.040.

Reddy, V.M., Nikhil, G.N., Venkata Mohan, S., Swamy, Y.V., Sarma, P.N., 2012. Pseudomonas otitidis as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. Bioresour. Technol. 123, 471–479. https://doi.org/10.1016/J.BIORTECH.2012.07.077.

Reis, M.A.M., Serafim, L.S., Lemos, P.C., Ramos, A.M., Aguiar, F.R., Van Loosdrecht, M.C. M., 2003. Production of polyhydroxyalkanoates by mixed microbial cultures. Bioprocess Biosyst. Eng. 25, 377–385. https://doi.org/10.1007/s00449-003-0322-4.

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. 102, 4499–4510. https://doi.org/10.1007/s00253-018-8931-7.

Ribeiro, J.M., Conca, V., Santos, J.M.M., Dias, D.F.C., Sayi-Ucar, N., Frison, N., Oehmen, A., 2022. Expanding ASM models towards integrated processes for short-cut nitrogen removal and bioplastic recovery. Sci. Total Environ. 821, 153492 https://doi.org/10.1016/j.scitotenv.2022.153492.

Rivera-Terceros, P., Tito-Claros, E., Torrico, S., Carballo, S., Van-Thuoc, D., Quillaguamán, J., 2015. Production of poly(3-hydroxybutyrate) by Halomonas boliviensis in an air-lift reactor. J. Biol. Res. 22 https://doi.org/10.1186/s40709-015-0031-6.

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. Manag. 205, 215–230. https://doi.org/10.1016/j.jenvman.2017.09.083.

Rojas-Z, U., Fajardo-O, C., Moreno-Andrade, I., Monroy, O., 2021. Effect of the famine phase length on the properties of aerobic granular sludge treating greywater. Water Sci. Technol. 84, 906–916. https://doi.org/10.2166/wst.2021.275.

Sabapathy, P.C., Devaraj, S., Parthipan, A., Kathirvel, P., 2019. Polyhydroxyalkanoate production from statistically optimized media using rice mill effluent as sustainable substrate with an analysis on the biopolymer’s degradation potential. Int. J. Biol. Macromol. 126, 977–986. https://doi.org/10.1016/j.ijbiomac.2019.01.003.

Sánchez-Valencia, A.I., Rojas Zamora, U., Meraz Rodríguez, M., Álvarez Ramírez, J., Salazar Peláez, M.L., Fajardo Ortiz, C., 2021. Effect of C/N ratio on the PHA accumulation capability of microbial mixed culture fed with leachates from the organic fraction of municipal solid waste (OFMSW). J. Water Process Eng. 40, 101975 https://doi.org/10.1016/j.jwpe.2021.101975.

Saratale, R.G., Cho, S.K., Saratale, G.D., Kumar, M., Bharagava, R.N., Varjani, S., et al., 2021. An overview of recent advancements in microbial polyhydroxyalkanoates (Pha) production from dark fermentation acidogenic effluents: a path to an integrated bio-refinery. Polymers 13, 4297. https://doi.org/10.3390/polym13244297.

Sekoai, P.T., Ghimire, A., Ezeokoli, O.T., Rao, S., Ngan, W.Y., Habimana, O., et al., 2021. Valorization of volatile fatty acids from the dark fermentation waste Streams-a promising pathway for a biorefinery concept. Renew. Sust. Energ. Rev. 143, 110971 https://doi.org/10.1016/j.rser.2021.110971.

Sen, K.Y., Hussin, M.H., Siti, B., 2019. Biosynthesis of poly(3-hydroxybutyrate) (PHB) by Cupriavidus necator from various pretreated molasses as carbon source. Biocatal. Agric. Biotechnol. 17, 51–59. https://doi.org/10.1016/j.bcab.2018.11.006.

Serafim, L.S., Lemos, P.C., Oliveira, R., Reis, M.A.M., 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. Biotechnol. Bioeng. 87, 145–160. https://doi.org/10.1002/bit.20085.

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. 81, 615–628. https://doi.org/10.1007/s00253-008-1757-y.

Sharma, N., 2019. Polyhydroxybutyrate (PHB) production by bacteria and its application as biodegradable plastic in various industries. Acad. J. Polym. Sci. 2 https://doi.org/10.19080/ajop.2019.02.555587, 555586–003.

Sharmila, G.V., Tamilarasan, K., Kumar, D.M., Kumar, G., Varjani, S., Kumar, S.A., Banu, J.R., 2022. Trends in dark biohydrogen production strategy and linkages with transition towards low carbon economy: an outlook, cost-effectiveness, bottlenecks and future scope. Int. J. Hydrog. Energy. https://doi.org/10.1016/j.ijhydene.2021.12.139.

Shen, M.Y., Chu, C.Y., Sawatdeenarunat, C., Bhuyar, P., 2022. Production, downstream processing, and characterization of polyhydroxyalkanoates (PHAs) boosted by pyruvate supplement using mixed microbial culture (MMC) and organic wastewater. Biomass Convers. Biorefin. 1–9 https://doi.org/10.1007/s13399-021-02170-w.

Silva, F., Matos, M., Pereira, B., Ralo, C., Pequito, D., Marques, N., Carvalho, G., Reis, M. A.M., 2022. An integrated process for mixed culture production of 3-hydroxyhexanoate-rich polyhydroxyalkanoates from fruit waste. Chem. Eng. J. 427, 131908 https://doi.org/10.1016/j.cej.2021.131908.

Singh, M., Patel, S.K.S., Kalia, V.C., 2009. Bacillus subtilis as potential producer for polyhydroxyalkanoates. Microb. Cell Factories 9, 1–11. https://doi.org/10.1186/1475-2859-8-38/TABLES/2.

Singh, S., Sithole, B., Lekha, P., Permaul, K., Govinden, R., 2021. Optimization of cultivation medium and cyclic fed-batch fermentation strategy for enhanced polyhydroxyalkanoate production by bacillus thuringiensis using a glucose-rich hydrolyzate. Bioresour. Bioprocess 8, 1–17. https://doi.org/10.1186/s40643-021-00361-x.

Sitthikitpanya, N., Sittijunda, S., Khamtib, S., Reungsang, A., 2021. Co-generation of biohydrogen and biochemicals from co-digestion of Chlorella sp. Biomass hydrolysate with sugarcane leaf hydrolysate in an integrated circular biorefinery concept. Biotechnol. Biofuels 14, 197. https://doi.org/10.1186/s13068-021-02041-6.

Sohn, Y.J., Son, J., Jo, S.Y., Park, S.Y., Yoo, J.I., Baritugo, K.-A., Na, J.G., Choi, J.-I., Kim, H.T., Joo, J.C., Park, S.J., 2021. Chemoautotroph Cupriavidus necator as a potential game-changer for global warming and plastic waste problem: a review. Bioresour. Technol. 340, 125693 https://doi.org/10.1016/j.biortech.2021.125693.

Surendran, A., Lakshmanan, M., Chee, J.Y., Sulaiman, A.M., Thuoc, D.V., Sudesh, K., 2020. Can Polyhydroxyalkanoates be Produced Efficiently from Waste Plant and Animal Oils? Front. Bioeng. Biotechnol. 8, 169. https://doi.org/10.3389/fbioe.2020.00169.

Tamang, P., Arndt, C., Bruns-Hellberg, J., Nogueira, R., 2021. Polyhydroxyalkanoates production from industrial wastewaters using a mixed culture enriched with Thauera sp.: Inhibitory effect of the wastewater matrix. Environ. Technol. Innov. 21, 101328 https://doi.org/10.1016/j.eti.2020.101328.

Tao, W., Lv, L., Chen, G.Q., 2017. Engineering Halomonas species TD01 for enhanced polyhydroxyalkanoates synthesis via CRISPRi. Microb. Cell Factories 16, 1–10. https://doi.org/10.1186/s12934-017-0655-3.

Thomas, T., Sudesh, K., Bazire, A., Elain, A., Tan, H.T., Lim, H., Bruzaud, S., 2020. PHA production and PHA synthases of the halophilic bacterium Halomonas sp. SF2003. Bioengineering 27, 29. https://doi.org/10.3390/BIOENGINEERING7010029.

Turon, V., Trably, E., Fouilland, E., Steyer, J.P., 2016. Potentialities of dark fermentation effluents as substrates for microalgae growth: a review. Process Biochem. 51, 1843–1854. https://doi.org/10.1016/j.procbio.2016.03.018.

Tyagi, P., Sharma, A., 2021. Utilization of crude paper industry effluent for Polyhydroxyalkanoate (PHA) production. Environ. Technol. Innov. 23, 101692 https://doi.org/10.1016/j.eti.2021.101692.

Urtuvia, V., Villegas, P., González, M., Seeger, M., 2014. Bacterial production of the biodegradable plastics polyhydroxyalkanoates. Int. J. Biol. Macromol. 70, 208–213. https://doi.org/10.1016/j.ijbiomac.2014.06.001.

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. 58, 12149–12158. https://doi.org/10.1021/ACS.IECR.9B01831.

Vargas, A., Monta˜no, L., Amaya, R., 2014. Enhanced polyhydroxyalkanoate production from organic wastes via process control. Bioresour. Technol. 156, 248–255. https://doi.org/10.1016/J.BIORTECH.2014.01.045.

Verlinden, R.A.J., Hill, D.J., Kenward, M.A., Williams, C.D., Radecka, I., 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates. J. Appl. Microbiol. 102, 1437–1449. https://doi.org/10.1111/j.1365-2672.2007.03335.x.

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. New Biotechnol. 31, 289–296. https://doi.org/10.1016/j.nbt.2013.08.001.

Vu, D.H., Wainaina, S., Taherzadeh, M.J., Åkesson, D., Ferreira, J.A., 2021. Production of polyhydroxyalkanoates (PHAs) by Bacillus megaterium using food waste acidogenic fermentation-derived volatile fatty acids. Bioengineered 12, 2480–2498. https://doi.org/10.1080/21655979.2021.1935524.

Wang, Y.J., Hua, F.L., Tsang, Y.F., Chan, S.Y., Sin, S.N., Chua, H., Yu, P.H.F., Ren, N.Q., 2007. Synthesis of PHAs from waster under various C: N ratios. Bioresour. Technol. 98, 1690–1693. https://doi.org/10.1016/j.biortech.2006.05.039.

Wang, Y., Yin, J., Chen, G.Q., 2014. Polyhydroxyalkanoates, challenges and opportunities. Curr. Opin. Biotechnol. 30, 59–65. https://doi.org/10.1016/J.COPBIO.2014.06.001.

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 8, 273. 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. 112, 269–278. https://doi.org/10.1016/j.watres.2017.01.064.

Wang, K., Hobby, A.M., Chen, Y., Chio, A., Jenkins, B.M., Zhang, R., 2022a. Techno-economic analysis on an industrial-scale production system of polyhydroxyalkanoates (PHA) from cheese by-products by halophiles. Processes 10, 17. https://doi.org/10.3390/pr10010017.

Wang, Z., Zheng, Y., Ji, M., Zhang, X., Wang, H., Chen, Y., Wu, Q., Chen, G.-Q., 2022b. Hyperproduction of PHA copolymers containing high fractions of 4-hydroxybutyrate (4HB) by outer membrane-defected Halomonas bluephagenesis grown in bioreactors. Microb. Biotechnol. 15, 1586–1597. https://doi.org/10.1111/1751-7915.13999.

Woo, H.C., Kim, Y.H., 2019. Eco-efficient recovery of bio-based volatile C2–6 fatty acids. Biotechnol Biofuels 12, 92. https://doi.org/10.1186/s13068-019-1433-8.

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. 262, 121328 https://doi.org/10.1016/J.JCLEPRO.2020.121328.

Yan, Q., Zhao, M., Miao, H., Ruan, W., Song, R., 2010. Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae. Bioresour. Technol. 101, 4508–4512. https://doi.org/10.1016/j.biortech.2010.01.073.

Yu, J., 2001. Production of PHA from starchy wastewater via organic acids. J. Biotechnol. 86, 105–112. https://doi.org/10.1016/S0168-1656(00)00405-3.

Zhang, X., Lin, Y., Wu, Q., Wang, Y., Chen, G.Q., 2020. Synthetic biology and genome-editing tools for improving PHA metabolic engineering. Trends Biotechnol. 38, 689–700. https://doi.org/10.1016/J.TIBTECH.2019.10.006.

Zhao, L., Han, D., Yin, Z., Bao, M., Lu, J., 2019. Biohydrogen and polyhydroxyalkanoate production from original hydrolyzed polyacrylamide-containing wastewater. Bioresour. Technol. 287, 121404 https://doi.org/10.1016/j.biortech.2019.121404.

Zhou, W., Colpa, D.I., Geurkink, B., Euverink, G.J.W., Krooneman, J., 2022. The impact of carbon to nitrogen ratios and pH on the microbial prevalence and polyhydroxybutyrate production levels using a mixed microbial starter culture. Sci. Total Environ. 811, 152341 https://doi.org/10.1016/j.scitotenv.2021.152341.