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Bio-conversion of organic wastes towards polyhydroxyalkanoates

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ABSTRACT

The bio-manufacturing of products with substantial commercial value, particularly polyhydroxyalkanoates (PHA), using cost-effective carbon sources through microorganisms, has garnered heightened attention from both the scientific community and industry over the past few decades. Opting for industrial PHA production from various organic wastes, spanning industrial, agricultural, municipal, and food-based sources, emerges as a wiser choice. This strategy not only eases the burden of recycling organic waste and curbs environmental pollution but also trims down PHA production costs, rendering these materials more competitive in commercial markets. In addition, PHAs are a family of renewable, environmentally friendly, fully biodegradable and biocompatible polyesters with a multitude of applications. This review provides an overview of recent developments in PHA production from organic wastes. It covers the optimization of diverse metabolic pathways for producing various types of PHA from organic waste sources, pre-treatment and downstream processing for PHA using unrelated organic wastes, and challenges in industrial production of PHA using unrelated organic waste feedstocks and the challenges faced in industrial PHA production from organic wastes, along with potential solutions. Lastly, this study suggests underlying research endeavors aimed at further enhancing of the feasibility of industrial PHA production from organic wastes as an alternative to current petroleum-based plastics in the near future.

1. Introduction

Organic wastes, including food waste, municipal solid waste, industrial wastewater, agricultural waste and residues from the food and beverage industry, paper industry, agriculture, residences, and wastewater treatment plants, represent rich sources of carbon and nitrogen. These waste sources can be utilized as cost-effective and environmentally friendly substrates for the production of bio-products through the use of recombinant microbes.[1–4] Due to the rapid growth of population and the blooming development of the economy, the volume of organic waste has surged,[5] encompassing agricultural, industrial and municipal solid wastes (see Fig. 1). According to statistics, Europe consumed over 17 million tons of edible oil in 2000.[6] Such extensive consumption results in a significant volume of waste oil, which cannot be effectively managed, leading to substantial soil pollution and economic losses. Furthermore, the global annual production and consumption of petroleum-based plastics exceed 140 million tons annually, contributing significantly to fossil fuel consumption, reaching up to 150 million tons petroleum. Based on an incomplete statistics for the present year (2020), organic wastes is primarily managed through various methods, including recycling, incineration, landfill composting, and anaerobic digestion, among others.[7] However, these treatment approaches do not provide sustainable solutions for most organic wastes like cooking oil, volatile fatty acids (VFAs), cellulose and plastics.

PHA is a family of polyesters naturally synthesized by numerous bacterial and archaeal species under nutrient unbalance conditions, which was firstly discovered in *Bacillus megaterium* by Maurice Lemoigne in 1926.[8–11] Due to the high similarity of mechanical properties

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compared to petrol-based plastics, as well as high-performing biodegradability and biocompatibility, PHAs has been widely studied and gradually used as an eco-friendly biomaterial with an extensive range of applications.[8,12–14] Based on the intensive efforts in previous studies, different types of PHA have been discovered and produced by engineered microbes with over 150 reported PHA monomers.[15] According to the number of carbon atoms of various monomers, PHA polymers can be divided into three categories: short-chain length PHA (scl-PHA) containing monomers with carbon atoms less than 6, such as poly-3-hydroxybutyrate (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P34HB); medium-chain length PHA (mcl-PHA) consist of monomers with 6–14 carbon atoms (e.g., poly-3-hydroxyhexanoates (PHHx), poly-3-hydroxyoctanoates (PHO), poly-3-hydroxydecanoates (PHD); long-chain length PHA (lcl-PHA) composed of monomers containing more than 14 carbon atoms.[16,17] To date, numerous bacterial species (e. g., *Escherichia coli*,[18] *Pseudomonas putida*,[19] *Halomonas*,[20] *Rastonia eutropha*,[21,22] *Haloferax mediterranei*,[23–25] *Salinivibrio* sp.,[26–28] etc.) were developed for effective production of PHA from various carbon sources, such as glucose, glycerol, VFAs, etc. In general, the most-studied biosynthetic pathway of PHA involves three key enzymes, β-ketoacyl-CoA thiolase encoded by *phaA*, acteoacetyl-CoA reductase encoded by *phaB*, and PHA synthase encoded by *phaC*,[29] converting acetyl-CoA, an important metabolite derived from substrate metabolism, into PHB granules accumulated by different microbes.[30]

Although PHA has excellent material properties, the commercial price is much higher than that of petroleum-based plastic due to the high cost of energy and feedstocks consumption during fermentation, and downstream processing.[31,32] Therefore, efforts have been made to obtain PHA production using organic wastes as carbon sources based on engineered microbes, which not only provides an alternative solution for organic wastes treatment, but also significantly reduce the cost of PHA biomanufacturing. There are a number of complex organic wastes that have been developed as carbon sources for PHA synthesis by recombinant microorganism, such as organic waste from biodiesel production,[33] buttermilk isolated from dairy waste,[34] sugarcane bagasse,[35] fatty acids-containing waste,[36,37] traditional plastic waste,[38] lignocellulosic feedstocks[39] and others (Fig. 1, Table 1). The PHA production from organic wastes has opened a proven possibility of waste biomass bioconversion and biorefinery, however, it does come with hurdles. Firstly, the composition of organic wastes such as crude glycerol[40] is complex and variable. These materials may contain components that certain microorganisms cannot utilize and, in some instances, may even act as inhibitors, potentially affecting the fermentative production of PHA. Secondly, complicated pre-treatment process is generally required, since most organic wastes, such lignocellulose, cannot be used directly and efficiently by most microbes, as well as their recombinants. For instance, many industrially pretreated wastes contain toxic components such as furfuraldehyde, VFAs, which are not suitable carbon sources for microbes to grow with effective PHA accumulation.[41]

Microorganisms accumulate PHA in cells by metabolizing carbon substrate in the form of sugars, wastes or lipids contained in organic wastes. PHA is similar to traditional plastics and has the advantage of being environment-friendly, biocompatible and biodegradable. It has a wide application prospects in food, medicine, packaging, etc. used in daily life.[51] Since PHA offers a broad scope of application scenarios with considerable market demands, however, the production cost ranges from 3.0 to 8.5 €/kg depending on the polymer composition, while the cost of traditional petroleum based plastics is about 1.0–2.0 €/kg.[52] The elevated production cost is a significant barrier, and addressing this

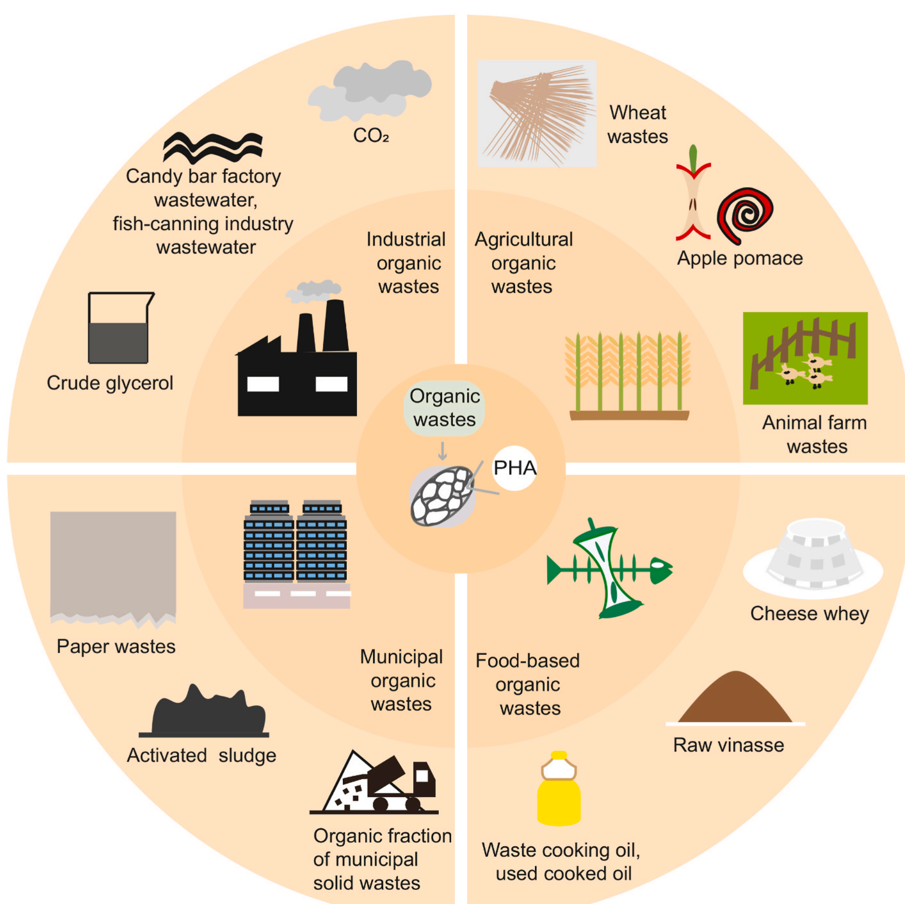

Fig. 1. Overview of diverse organic wastes suitable for PHA production.

Table 1
Production of PHA using diverse carbon source from organic wastes.

Carbon Sources	Strains	PHA	Cell dry weight (g/L)	PHA content (%)	PHA productivity (g/L/h)	References
Rice bran	*E. coli* XL1-Blue (pCnCAB)	PHB	2.98 ± 0.01	90.1 ± 0.1	NA	21
Rice bran	*R. eutropha* 437–540 (pKM212-LdhA)	P(3HB-*co*-LA)	1.53 ± 0.03	35.8 ± 0.7	NA	21
Sunflower stalk	*R. eutropha* NCIMB11599 (pKM212-XylAB)	PHB	2.46 ± 0.04	84.96 ± 0.89	NA	22
Olive mill wastewater	*Haloferax mediterranei*	P(3HB- *co*-6.5 mol% 3HV)	10.0	43.0	NA	24
Waste fish oil and glycerol	*Salinivibrio* sp. M318	PHB	69.1	51.5	0.46	26
Acetate and butyrate	*Salinivibrio* spp. TGB19	PHB	8.17 ± 0.38	83.87 ± 1.80	0.14	27
Butyrate	*Marinobacterium sediminicola*	P(3HB- *co*-94.75 mol% 3HV	4.50 ± 0.23	74.99 ± 2.61	0.07	37
Wheat straw	*Burkholderia sacchari* DSM17165	PHB	146	72	1.6	42
Crude glycerol	*P. putida* KT2440	mcl-PHA	4.23 ± 0.11	34.5	NA	43
Crude glycerol	*P. putida* KT2440	mcl-PHA	49.5	27.9	0.23	44
Crude glycerol	*P. putida* KT2440Δ PhaZ	mcl-PHA	52.4	38.9	0.34	44
Waste vegetable oil	*P. putida* KT2440Δ tatA	mcl-PHA	NA	38.3 ± 3.1	0.027	45
High-fructose corn syrup	*Halomonas* sp. YLGW01	PHB	NA	67.39 ± 0.34	0.11	46
P-coumaric acid	*P. putida* AG2162	mcl-PHA	1.76	54.2	0.011	47
Lignin containing stream	*P. putida* AG2162	mcl-PHA	0.65	17.7	0.0015	47
Xylose	*E. coli GPT2000*	PHB	5.49 ± 0.31	17.25 ± 0.36	NA	48
Fish-canning wastewater	Microbial consortium	PHB, PHV	NA	8.35	0.010	49
Acetate	*E. coli* JM109 (p68-pct-CAB+pMCS-pta-ackA-prpP)	P(3HB- *co*-10.37 mol% 3HV)	3.15	34.77	NA	50

NA, not available.

issue is becoming a crucial challenge for the commercial utilization of PHA. Utilizing organic wastes, such as straw and wheat bran from agricultural production, molasses and bagasse from sugar production, crude glycerol from the biodiesel industry, as well as VFAs, whey and other waste effluents from industrial production, not only can recycle the waste biomass, but also reduce over 50 % substrate costs for PHA production.[53] Therefore, it is of great significance to explore organic waste in terms of an economically feasible, stable and efficient synthesis of PHA.

Recently, many studies have made efforts to develop different strategies for effective bioconversion of different types of organic wastes to PHA. In this review, we summarize recent developments focusing on various PHA synthesis from organic wastes. Besides, solutions for improved utilization of organic wastes including waste biomass pretreatment, cell factory engineering, etc. were also proposed in this study aiming to achieve efficient production yield of different types of PHA from waste-derived substrates. Moreover, the fermentation process optimization, challenges and possible solutions for industrial scale PHA production based on organic wastes were also discussed.

2. Advances in microbial production of PHA from organic wastes

Recently, a number of PHA polymers have been synthesized by genetically engineered microorganisms using organic wastes as carbon source, such as industrial organic wastes including biodiesel waste,[54,55] agricultural organic wastes derived from wheat and[42] rice bran,[21] and food-based organic wastes like waste cooking oil[45] (Fig. 1, Table 1). However, using organic waste as substrate for PHA production still remains challenge due to the difficulties of complex pretreatment and ineffective bioconversion metabolism. Therefore, many strategies have been developed to achieve enhanced bioconversion of organic wastes for customized products, including growth medium modification,[56] fermentation process optimization,[57] enhancement of stress resistance, bypasses deletion[58] and reinforcement of target fluxes.[58,59] Besides, omics profiling such as genomics, proteomics and transcriptomics have also been used to direct microbial cell factory engineering for improved efficiency of organic wastes utilization and PHA synthesis.

Pseudomonas putida (*P. putida*) is the best-known and most-studied producer of mcl-PHA.[60] The type of nutrient limitation has an effect on genes expression involved in pathways related to PHA synthesis, energy production and conversion, carbohydrate transport, synthesis of amino acids, nitrogen scavenging, cellular processing, transcriptional regulation and so on.[61] Therefore, the carbon-to-nitrogen ratio can influence mcl-PHA accumulation in *P. putida*.[62] A previous study demonstrated that nitrogen limitation during the cultivation of *P. putida* Gl01 on oleic acid and sodium gluconate had no substantially influence on CDW accumulation, however, could significantly improve mcl-PHA biosynthesis.[63] Interestingly, for some cases of *P. putida* mutants grown on gluconate, nitrogen limitation had little effect on mcl-PHA production while enhanced mcl-PHA accumulation was observed when the carbon source was changed.[64]

Recently, many studies have tried to genetically optimize the metabolic pathways related to utilization of organic waste derived substrates. Crude glycerol, one of the well-studied waste products from biodiesel processing, has been employed for the cost-effective production of PHA. It serves as a low-cost substrate for biopolymer production by certain microorganisms. Under nitrogen limiting conditions, the titer of PHA(wt %) by engineered *P. putida* KT2440 grown on raw glycerol as the only carbon source can reached up to 47 % by knocking out the gene *phaZ* encoding PHA depolymerase,[43] which resulted in a higher PHA titer (12.7 gPHA/L, 27.2 % of the cell dry weight (CDW)) compared to the wild-type strain (9.7 gPHA/L, 21.4 % of the CDW) after 60 h fed-batch cultivation under nitrogen limitation with crude glycerol as carbon source. Furthermore, dissolved-oxygen-stat (DO-Stat) feeding strategy under nitrogen limitation was employed to generate higher mcl-PHA cell content and productivity, reaching 38.9 wt% (52.4 g/L) and 0.34 g/L/h, respectively, which was 48 wt% higher than the wild-type strain (KT2440), during a 60 h fermentation process using crude glycerol as carbon source.[44] Moreover, inactivation of the tricarboxylate (TCA) transport system via knocking out *tctA* gene showed proven effect on enhancing the accumulation of mcl-PHA by recombinant *P. putida* KT2440.[45] In addition to crude glycerol, lignocellulosic biomass mainly containing glucose, xylose and toxic compounds was also developed as low-cost carbon sources for PHA synthesis by engineered microbes. *P. putida* AG2162 derived from *P. putida* KT2440 with *phaZ* gene, *fadBA*$_1$

and *fadBA*$_2$ genes involved in fatty acid β-oxidation konckout has been engineered to produce mcl-PHA effectively from lignin-derived compound, p-coumaric acid, by overexpressing *phaG*, *alkK*, *phaC*$_1$ and *phaC*$_2$ genes. The mcl-PHA yield and titer reached 17.7 % and 166 mg/L, respectively, after 78 h of cultivation.[47]

Non-PHA producing microorganisms are able to synthesize PHA by overexpressing genes related to PHA synthesis, such as *phaCAB* operon from *Ralstonia eutropha*.[65] *Escherichia coli*, the most-studied model bacterium has been engineered to produce various PHA production using organic wastes as carbon source. To date, engineered *E. coli* are able to effectively use crude glycerol,[66] xylose,[67] etc. as the sole carbon source to produce PHA. In order to improve the bio-utilization of different carbon sources, the non-oxidative glycolysis (NOG) pathway was introduced in *E. coli* to improve the PHA yield by 26.3 % and 43.3 % for xylose and glycerol, respectively, compared to the start host.[67] *E. coli* GPT2000, which is derived from *E. coli* GPT1002 with chromosomal integration of *phaCAB*, accumulated 14.4 g/L L-tryptophan and 9.7 % PHB (w/w) after fed batch cultivation grown on xylose only.[48] *E. coli* JM109 (DE3) harboring a high-activity L-aspartate decarboxylase encoded by *panD* gene was able to accumulate 10.2 g/L poly-3-hydroxypropionate (P3HP) under aerobic fed-batch fermentation condition.[68] Moreover, co-culture of engineered *E. coli* Δ4D (T3), which can convert sugars into PHA-precursors such as acetic acid and free fatty acids, and *P. putida* KTΔAB (p2-acs-phaJ), which can produce mcl-PHA from glucose, acetic acid and free fatty acids, has been demonstrated to produce mcl-PHA using a mixed substrate of glucose and xylose, the main components of lignocellulosic hydrolysates, which resulted in a maximum titer of 1.32 g/L leveraging substrate competitive feeding strategy.[69]

Screening of novel strains with natural metabolic capability of organic waste was also an effective solution for PHA synthesis. Muang Wong et al., isolated four *Pseudomonas* strains (ASC1, ASC2, ASC3, ASC4) from soils in Thailand, which are able to efficiently use crude glycerol as a carbon source for the production of mcl-PHA consisting of 3-hydroxyoctanoate (3HO) and 3-hydroxy-5-cis-dodecanoate (3H5DD) monomers. Notably, *Pseudomonas* sp. ASC2 showed the highest mcl-PHA accumulation performance after 36 h batch cultivation, resulting in a total CDW of 32.3 ± 0.3 g/L containing 61.8 ± 3.3 wt % mcl-PHA, respectively.[70] Besides, a newly isolated *Halomonas* sp. YLGW01 could produce 7.95 ± 0.11 g/L PHB after 72 h of cultivation in sea water-based medium using high-fructose corn syrup as carbon source, which mainly contains fructose, glucose and sucrose.[46] And several species of marine bacteria from the genus *Marinobacterium* (*M. nitratireducens*, *M. sediminicola*, and *M. zhoushanense*) were found to utilize VFAs as the carbon source for PHA production. Notably, when valerate was used as the substrate, *M. nitratireducens* resulted in a PHBV titer of 2.45 g/L, with a 3HV monomer content of 99.13 mol%.[37] Specifically, many metabolic pathways have been identified and constructed for diverse PHA synthesis from different organic wastes derived compounds, such as xylose, glucose, acetate, fatty acids, glycerol and so on, after pretreatment processing (Fig. 2).[71]

In addition, studying mixed microbial consortia (MMC) is also an

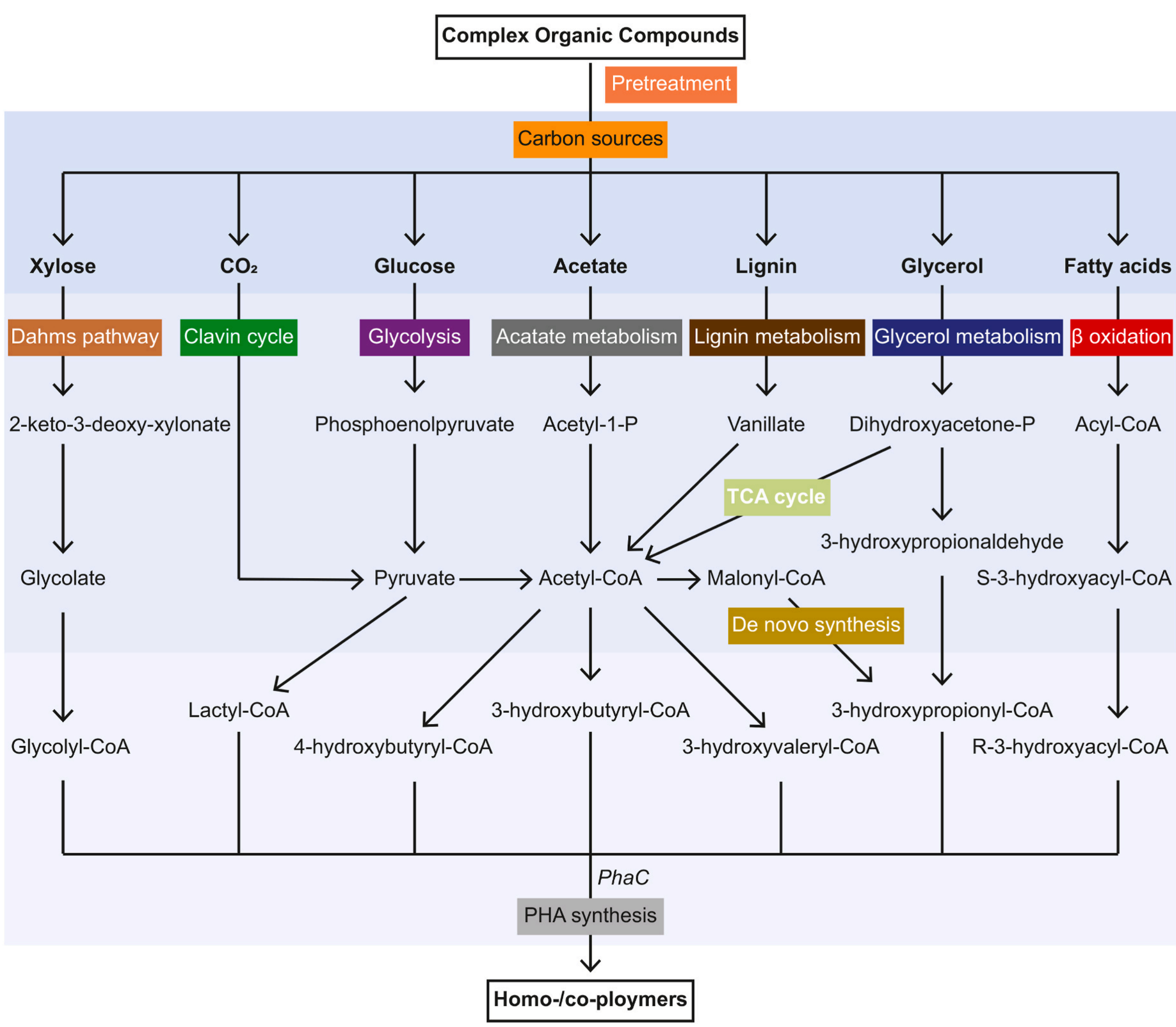

Fig. 2. Metabolic pathways for PHA synthesis from organic waste carbon sources.

alternative scenario for PHA production from organic wastes, which shows several advantages including non-sterile fermentation process, high tolerance and adaptability to waste substrates.[72,73] MMC-based PHA production generally can be obtained by performing three-step process, including feedstock and the acidogenic fermentation step (obtaining useable substrate streams for microorganisms growth and PHA accumulation), enrichment of biomass production (achieving concentrated organic waste-derived carbon sources and high cell density of PHA-producing cells) and effective accumulation of PHA.[74] Among them, enrichment process is one of the key processes for achieving high bioconversion rates of PHA from organic wastes. Anna Burniol-Figols et al., performed a novel enrichment strategy to obtain PHA-forming MMC based on open-culture processes using fermented crude glycerol as substrates, resulting in a high PHA production rate reaching up to 0.41 g PHA.L^{-1}.h^{-1} [73]. Dongna Li et al., used palm oil and wastewater-derived hydrolysate as carbon sources to obtain PHA through MMC grown in activated sludge, yielding high PHA content (30.5 wt%) and volumetric yield (0.372 g PHA-COD/g COD).[75] Moreover, 8-day enrichment of pig farm sludge using lactic acid fermentation broth was employed to achieve enhanced MMC PHA production based on MMC, achieving PHA yield of 0.57 g PHA-COD/g COD and PHA content of 50.1 wt%.[76] Particularly, researchers have made great efforts to improve MMC-based PHA production from organic waste streams incorporation of a diversity of environmental and biological technologies powered by synthetic biology.

3. Challenges and potential solutions for industrial PHA production from organic wastes

At present, pilot and industrial scale PHA manufacturers are active all over the world, including companies such as Medpha (Zhuhai, China),[77] PHAbuilder (Beijing, China),[78] Biomer (Germany),[11] Keneka (Janpan)[79] and so on. However, organic wastes are rarely utilized to produce PHA on such a large scale because of many unsolved challenges, such as complex pretreatment process, poor cell growth on organic waste feedstocks, low conversion efficiency of raw material to PHA, and an expensive downstream separation and purification process.

The complex pretreatment process mainly involves dilution of organic matter, increasing the available carbon sources, pH and temperature control, sterilization of waste materials, removal of suspended solids and cell growth inhibited compounds.[31] Some organic wastes can be easily transformed into PHA after physico-chemical or biological pretreatments. For example, VFAs produced from fish-canning wastewater after acidogenic fermentation was utilized as a substrate for PHA accumulation,[49] pretreated Toma cheese whey was used for PHA synthesis by a mixed microbial culture.[80] However, most pretreated organic wastes with complex composition can still contain toxic substances that inhibit cell growth. Therefore, the extra removal of condensed impurity and pigment from organic wastes will also increase the cost of PHA production. Besides, most microorganisms can secrete organic acids as byproducts, which act as cell growth inhibitors, during the fed-batch fermentation,[81] resulting in poor cell growth and PHA accumulation. Moreover, overexpression of metabolic pathways for enhanced carbon sources utilization and PHA synthesis may have a negative impact on cell growth,[82] leading to low PHA titer, yield and productivity. Regarding the downstream process, PHA purification generally requires three steps: cell pellet separation, cell lysis, PHA extraction and purification, and drying. Specifically, recombinant cells with PHA accumulation were harvested by centrifuge after batch- or fed-batch fermentation.[83,84] Generally, several approaches for intracellular PHA extraction after high cell-density fermentation. To date, PHA extraction via non-halogenated solvents or aqueous-phase cell lysis are commonly used methods for laboratory- and industry-scale PHA extraction and purification, which can generate PHA productions of high purity and lower cost, respectively.[85–87] However, there are still some drawbacks, including the high cost associated with the solvent-based PHA extraction process, and the suboptimal quality of PHA production using the aqueous-phase extraction process. Thus, developing effective PHA extraction and purification process of strong economical and technical feasibility is crucial for achieving low-cost and eco-friendly PHA production from organic wastes.

Designing novel metabolic pathways is a widely used approach to achieve enhanced PHA production from organic wastes (Fig. 3). Generally, three major strategies have been developed for optimized PHA accumulation capability of microorganisms from organic wastes. The first strategy is screening for high-performing microorganisms able to utilize organic wastes with strong PHA accumulation capacity. Wild-type *Salinivibrio* sp. M318, isolating from fermenting shrimp paste, achieved high CDW of 69.1 g/L containing 51.5 wt% PHB after 78 h fed-batch cultivation using fish sauce and mixtures of waste fish oil and glycerol as nitrogen and carbon sources, respectively, which was proved to be a promising chassis strain for PHA synthesis from aquaculture residues.[26] *Vibrio alginolyticus* LHF01 and *Halomonas* spp. TD01 even showed strong potentiality for PHA production using cheap waste glycerol from biodiesel, which are developed to be attractive chassis for PHA synthesis from diversified organic wastes due to its excellent performance of fast-growth, high salt tolerance enabling open nonsterile fermentation and wide substrate utilization.[20,88] The second strategy is to develop recombinant strains leveraging adaptive evolution and metabolic engineering approach with enhanced utilization efficiency of organic wastes based on natural PHA producers. *C. necator* DSM 545 is a well-known PHA producer, which normally is not able to utilize lactose. After insertion of *lacZ* and *lacI* genes from *E. coli* and deletion of $phaZ_1$, $phaZ_3$ genes, the resultant recombinant *C. necator* mRePT was able to grow on lactose and whey permeate with an improved PHA production capability.[89] Recombinant *R. eutropha* NCIMB11599 harboring expression vessel that contains the *xylAB* genes from *E. coli* could synthesize PHB from xylose-rich sunflower stalk hydrolysate.[22] The third strategy is to rationally design model organisms for PHA synthesis using organic wastes. Recombinant *E. coli* JM109 (pBHR68 + pMCS-pta-ackA) with overexpression of *pta-ackA* operon encoding phosphotransacetylase and acetate kinase, respectively, and *phaCAB* operon encoding PHB synthesis enzymes could produce PHB using acetate, the major fermentation product during anaerobic digestion of organic wastes, as a main carbon source.[50]

Pretreatment development of organic wastes can significantly improve the economy and efficiency of PHA synthesis (Fig. 3), because many raw organic wastes cannot be directly utilized by microorganisms and several potentially toxic components such as furfuraldehyde, heavy metals, pathogens, etc. are present in organic wastes streams.[4,90] There are several pretreatment technologies available for organic wastes, such as physicochemical treatment and biological fermentation. Generally, physicochemical pretreatment such as hydrothermal treatment[91] and sulfate reduction[92] for organic waste streams is widely used method, however, there are some toxic and harmful byproducts retained in the pre-treatment hydrolysate, such as solvent residues (trace impurities, etc.), significantly affecting cell growth and PHA accumulation.[93–95] To address this problem, green solvents such as γ-valerolactone[96] and novel strategies such as synchrotron radiation-based fourier transform infrared analysis integrated with μ-X-ray fluorescence[97] were employed to alleviate toxic and harmful byproducts. Recently, biological pretreatment, including anaeobic digestion (AD), enzymatic degradation and so on, are developed and widely used because of their environmentally friendly process of low cost and less harmful by-products accumulation.[98–100] Anaeobic digestion, comprising four stages — hydrolysis, acidogenesis, acetogenesis and methanogenesis, guided by microbial communities — is a widely used biological fermentation technology for the pre-treatment of organic waste streams. This includes food wastes, lignocellulosic biomass and residues, energy crops and organic components of municipal solid wastes.[101,102] Acidogenesis is the most important stage that determines the metabolic activity of biomass conversion and transforms the hydrolytic products into VFAs, which can

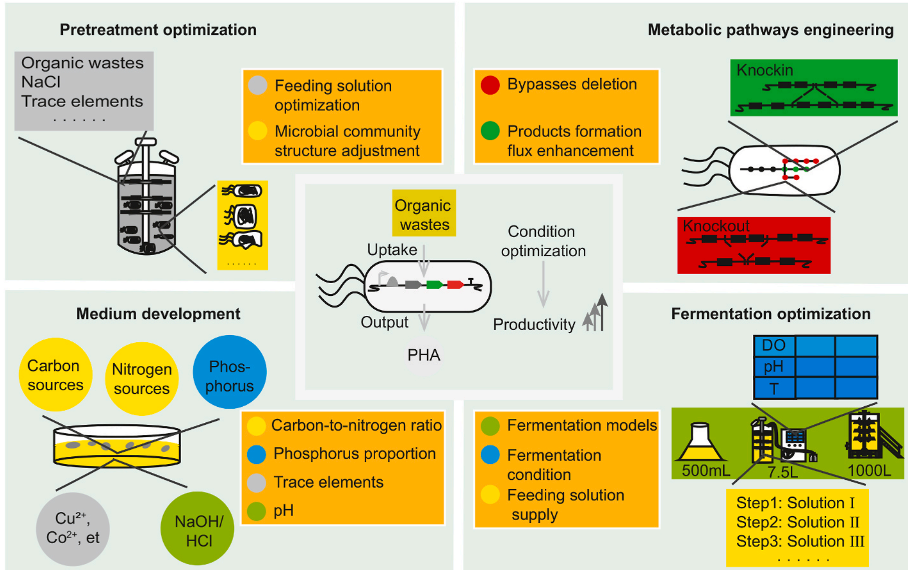

Fig. 3. Schema of various approaches to improve the bio-conversion rate from organic wastes towards PHA production.

also be diverted to serve as carbon source for further PHA production.[103] However, there are still a lot of challenges of AD systems, such as low buffer capacity and process instability. Hence, engineering the microbial consortia is an effective method to improve the pretreated performance of AD system.

Finally, developing extremophiles as microbial cell factories is one of the most suitable strategies to achieve cost-effective PHA production using organic wastes as carbon sources (Fig. 3). Extremophiles can grow at extreme conditions of high pH, salt concentrations or temperatures that most microorganisms cannot survive, thus reducing contamination risk. Besides, many extremophiles have strong resistance to toxic compounds and natural digestion capacity of different substrate in organic waste hydrolysate. In previous study, it has been demonstrated that recombinant *Halomonas campaniensis* LS21 strain can be continuously grown at non sterile conditions for over two months under conditions of 27 g/L NaCl, high pH at 10 and 37 °C without contamination by other bacteria, resulting in approximately 70 wt% PHB accumulation.[104] Moreover, *Halomonas* TD01 was engineered to use starch and fatty acids, respectively, as sole carbon source to produce PHA and value-added chemicals, which demonstrated proven foundations for bio-conversion of starch- and fatty acid enriched organic wastes.[105] Therefore, the utilization efficiency and economy of organic wastes can be potentially improved by coupling open unsterile fermentation process and coarsely organic wastes pretreatment process based on genetically modified Halophiles with enhanced substrate metabolism and resistance.

4. Conclusion

As an alternative candidate for replacing petroleum-based plastics, PHA offers a wide range of applications for consumers and industry. Comparison to petroleum-based plastics, industrial PHA production from pretreated organic waste feedstocks shows several advantages, such as good economic efficiency, eco-friendly process, and excellent sustainability without competitive consumption of food and farmland resources. To date, engineering metabolic pathways related to organic waste substrates digestion towards diverse PHA polymers synthesis have been intensively studied. Many achievements have been made at research level based on recombinant PHA producing strains. However, due to the complexity and high cost of organic waste pretreatment, and low conversion rate of organic waste derived substrates, the output-to-benefit ratio for PHA production is tepid. Therefore, there are still a lot of space for cost-effective PHA synthesis from organic wastes leveraging metabolic engineering approaches and high-performing chassis screening and development in corporation of pretreatment process optimization.

Credit author statement

The listed authors participated in the creation of this study in the following ways: Zhe-Yi Kuang, Hao Yang and Shi-Wei Shen wrote the manuscript and drafted the table and figures. Jian-Wen Ye, Hai-Tao Yue and Markus Neureiter proposed the idea and revised the manuscript. Yi-Na Lin revised the manuscript with minor comments and suggestions. Zhe-Yi Kuang and Shu-Wen Sun drafted and revised the Table. All authors read and approved the manuscript. Zhe-Yi Kuang, Hao Yang and Shi-Wei Shen contributed equally in this paper.

Declaration of competing interest

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

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