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The current application of commonly available fruit wastes for the synthesis of polyhydroxyalkanoates from bacteria

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The rapid depletion of fossil fuel and its hazardous effect on the environment encourages research on the synthesis of biobased plastics such as polyhydroxyalkanoates (PHAs) for the replacement of traditional plastics. Polyhydroxyalkanoates (PHAs) are intracellularly synthesized biopolymers that are non-toxic and biologically degradable in nature. Their physicochemical and mechanical properties are mostly similar to petrochemically derived plastics. A major limitation of the commercialization of polyhydroxyalkanoates is the high cost in comparison with petroleum-derived polymers. A lot of research is in progress towards searching for the cheapest carbon source for the culture of bacteria. Among various carbon sources available, biosynthesis of PHA from fruit wastes is still in its initial stage and several challenges remain to be resolved. In order to increase the availability of fermentable sugars and increase microbial intake, screening of more fruit waste materials and substrate pre-treatment procedures must be improved. For PHA recovery and purification, the development of effective and economical downstream processing methods is an additional area that needs focus. The economic feasibility of PHA manufacturing might be significantly increased by advancements in this area, as these processes now account for a significant amount of the total production cost. The systematic screening of various fruit waste products to determine which have the greatest potential for PHA generation should be the main focus of future studies. Furthermore, improvements in genetic engineering and the optimization of the metabolic pathways of microorganisms that produce PHA may increase production and lower expenses. This article presents a thorough analysis of the value-adding of different kinds of fruit wastes for the production of biopolymers, stressing the various strategies used thus far, their drawbacks, and possible future development paths. The large-scale synthesis of PHAs from fruit waste may prove to be a sustainable and profitable way to lessen the environmental effects of conventional plastics by tackling current issues and utilizing cutting-edge technologies.

Keywords: biopolymer; polyhydroxyalkanoates; fruit waste; bacterial fermentation; pretreatment methods.

Introduction

Overexploitation of non-renewable fossil fuel resources by the mining industries as well as the environmental and economic problems that arise from conventional petroleum-based plastics leads to the advancement of research pursuing material production from renewable resources by the green process. Furthermore, plastic is an unavoidable evil in our life due to its wide range of applications, mainly in packaging (Groh et al., 2019). Petrochemically derived plastics are not easily degraded and due to this accumulate in varied environments and create many serious ecological problems. During their decomposition, plastics break down into microplastics (MPs), which can easily enter the bodies of animals through food or water and transfer to other organisms via the food chain (Zhao et al., 2017). Worldwide plastic production is continuously increasing and it reached 359 million tons in 2018, which is 155 million tons more compared to 2002. Additionally, according to the Central Pollution Control Board (CPCB Report 2018-19), India generated approximately 3.3 million metric tons (which means 9,200 tons per day) of plastics in 2018–2019 (Kaushik et al., 2023). The Indian Union Ministry of Environment, Forecast and Climate Change (MoEFCC) issued a draft of Plastic Waste Management Rules, 2021, which imposed a ban on manufacturing, importing, stocking, distribution, sale, and use of single-use plastic from 1 January 2022. This rule was commonly called Plastic Waste Management Rules, 2021, and it shall be effectively implemented from when published in the Official Gazette. Therefore, it is necessary to replace synthetic polymers with eco-friendly alternatives. It is important to reduce environmental pollution by using renewable resources. This is a huge challenge because this non-renewable energy, water utilization and CO_2 emission need to be reduced and material needed to be recircularized (Mahjoub & Domscheit, 2020). Furthermore, biobased replacement of petrochemically derived polymers that are microbially synthesized by using renewable resources as a sole carbon source can reduce environmental pollution. The main properties of these biopolymers are that they are easy to recycle and become biologically degraded in the environment after their useful life (Sheldon & Norton, 2020). Among the various groups of polymers, polyhydroxyalkanoates (PHAs) have attracted much attention as they are naturally created for the accumulation of carbon and energy in discrete granules in the cytoplasm of bacteria (Bonartsev et al., 2019). PHAs are biologically synthesized polymers and have the potential to replace some commonly used petrochemical-based plastics (Dietrich et al., 2017). PHAs are hydrophobic storage granules that biosynthesize in the cytoplasm and are known as carbonosomes. They constitute up to 90% of the dry cell weight of the bacteria. Based on synthesizing bacteria and types of PHA, the size of PHAs ranges from 0.5 to 1.0 µm (Koller et al., 2017). PHAs are soluble in chlorinated hydrocarbons and chloroform with low resistance to acids and bases. Their main properties are resistance to hydrolytic degradation and UV radiation, and that they are insoluble in water and non-toxic (Sharma et al., 2021). PHAs have gained much attention in industries nowadays due to their biocompatibility, biodegradability, high performance and green credentials. The bioplastic market is growing at a steady rate as in 2017 commercially synthesized PHAs amounted to 2.05 million tons and it is also expected that the global PHA market was expected to increase to 98 million tons in 2024 (Ranganathan et al., 2020).

The main problem behind the commercialization of PHAs is the production cost, which is higher than conventionally derived plastics such as polyethylene and polypropylene. The high production cost of PHAs is mainly due to the major carbon source used for feeding the bacteria (Vicente et al., 2023). To reduce the cost of PHA biosynthesis, a considerable amount of research has been done using industrial waste as a sole carbon source for the microbial fermentation process.

On the other hand, microbially derived PHA biosynthesis from waste carbon sources could also reduce the cost of waste disposal (Amaro et al., 2019). Today, many biomass by-products are wasted while they can be used as an efficient carbon source (Antar et al., 2021). A lot of waste from fruit residues is generated every year after industrial processing. This includes apples, mangoes, grapes, citrus, bananas etc. These residues contain high amounts of fermentable sugars such as glucose, fructose and sucrose, which can be further used for the culture of bacteria (Luzón-Quintana et al., 2021). This review mainly focuses on PHA synthesis from different types of fruit wastes, its biosynthetic pathways, different types of pretreatment processes and PHA yields concerning biomass concentration.

Structure of polyhydroxyalkanoates (PHA)

PHA congeners can be found in about 150 distinct forms. Figure 1 illustrates the general structure of PHA. PHA can be named based on the type of R group and the number of repeating units of PHA monomer (n) viz; poly-3-hydroxy propionate (if n = 1 and R = hydrogen); poly-3-hydroxybutyrate (if n = 1 and R = methyl); poly-3-hydroxyvalerate (if n = 1 and R = ethyl); poly-3-hydroxyhexanoates (if n = 1 and R = propyl); poly-3-hydroxyoctanoate (if n = 1 and R = pentyl); poly-3-hydroxydodecanoate (if n = 1 and R = nonyl); poly-3-hydroxybutyrate (if n = 2 and R = hydrogen); poly-5-hydroxyvalerate (if n = 3 and R = hydrogen). Polyhydroxyoctanoates (PHO) is the name of the polymer if R = C_3H_7, and so on.

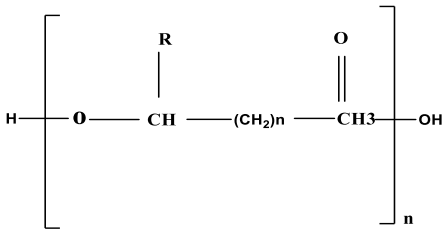

Fig. 1. Structure of polyhydroxyalkanoates

Classification of PHA

According to the number of carbons in the side chains, PHAs are classified into three classes, short chain length PHA (scl-PHA) having less than 5 carbon atoms (for example; 3-hydroxy valerate and 3-hydroxybutyrate), medium chain length PHA (mcl-PHA), which includes 5–14 carbon atoms (for example; 3-hydroxy decanoate, 3-octanoate and 3-hydroxy hexanoate) and long chain length PHA (lcl-PHA), which has more than 14 carbon atoms but are uncommon and less studied (Fig. 2).

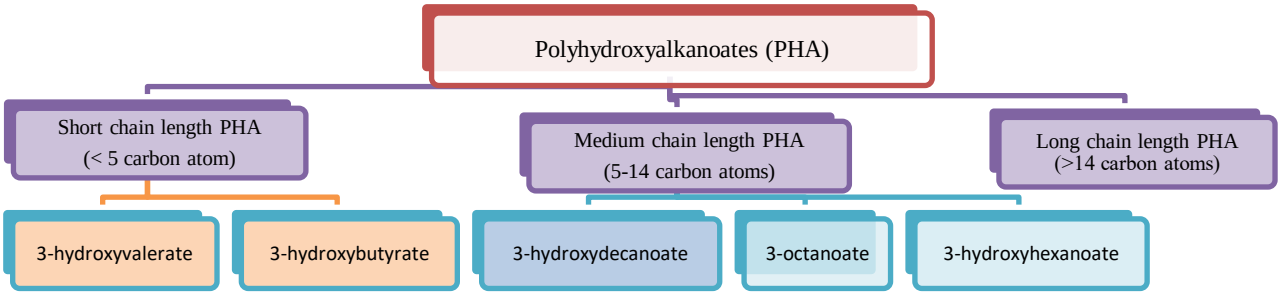

Fig. 2. General classification of polyhydroxyalkanoates

Properties of polyhydroxyalkanoates

PHAs are biodegradable, biocompatible, and good at mechanical and thermal processing. PHAs have distinct properties and chemical compositions (Fig. 3) as homo or copolymers as a result of the structural variations in their constituent monomers (Muneer et al., 2020). Similar to polypropylene, PHAs are resistant to moisture and acquire excellent barrier properties. PHAs are also insoluble in water, resistant to hydrolytic attack, and UV-resistant, and they sink in water, making it easier for anaerobic biodegradation to take place in the sediments. They also behave as piezoelectric materials because they can generate an electric charge after application of mechanical stress. Additionally, PHAs possess chiral molecules, and their degradation is primarily determined by their type and composition (Pryadko et al., 2021). In this manner, the biodegradation of PHAs is impacted by the kind and organization of the polymer, ecological circumstances and the type of microorganisms (various microorganisms produce different PHA-depolymerases to corrupt PHAs). Chloroform and other chlorinated solvents dissolve PHAs. Their melting temperature ranges from 40 to 180 °C, while their glass transition temperature ranges from –50 to 4 °C (Ranganadhareddy & Chandrsekhar, 2022). The rate of water vapor transfer, oxygen transmission, tensile strength, Young's modulus, and thermodegradation temperature are all influenced by the kind of polymer that is generated and the makeup of the monomeric unit. Current commercially available PHAs have low tensile strength and low elongation at break, which ranges from 28–40 Mpa and 2–8% respectively (Muthuraj et al., 2021).

Some common PHA-producing bacteria

Nowadays, more than hundreds of different bacterial species have been recognized as PHA producers since their discovery in the 20th century (Sedničková et al., 2018). Koller et al. observed that out of many identified bacteria, only a few are capable of a high rate of PHA synthesis. The PHA yield is generally depicted in percentage. Some of these bacteria are *Cupriavidus necator* (87%), *Pseudomonas aeruginosa* (55%), *Bacillus megaterium* (45%), *Pseudomonas putida* (37%), *Pseudomonas oleovorans* (13%) and *Pseudomonas fluorescens* (Nahar et al., 2019). There are some other bacteria also which can be used for the biosynthesis of polyhydroxyalkanoates. These are *Bacillus thermoamylovorans* (88%) (Sangkharak et al., 2021), *Bacillus siamensis* (82%), *B. subtilis* (75%) (Rayasam et al., 2020), *Burkholderia sacchari* (72%) (Kulkarni et al., 2015), *Paracoccus* sp. (72%) (Sawant et al., 2015), *Pseudomonas sacchari* (71%) (Dietrich et al., 2019), *Burkholderia thailandensis* (60%) (Kourmentza et al., 2018), *H. mediterranei* (56%) (Huang et al., 2006), *Burkholderia cepacian* (55%) (Pan et al., 2012), *Haloferax mediterranei* (55%) (Pais et al., 2016), *Bacillus thuringiensis* (52%) (Gowda & Shivakumar, 2014b), *Azotobacter beijerinickii* (48%) (Sathesh & Murugesan, 2010), *Pseudomonas chlororaphis* (48%) (Ruiz et al., 2019), and *Bacillus mycoides* (25%) (Narayanan et al., 2014).

Biosynthesis of polyhydroxyalkanoates

Different bacteria have the potential to synthesize different forms of PHA according to their nutritional requirement and environmental conditions (Cruz et al., 2022). Based on the nutritional requirement on the laboratory scale, Mohammadi and his coworkers classified PHA-producing bacteria into two groups ie; group A and group B. Group A bacteria synthesize PHA only in the excess of carbon source and limited nutrient conditions such as nitrogen, phosphorus, sulphur or magnesium, for example: *Ralstonia eutropha*, *Protomonas extorquens* and *P. oleovorans*. Group B includes those bacteria that synthesize and accumulate PHA without any nutritional limitations but in

the presence of a carbon source during its growth phase, for example: *Alcaligenes latus* and recombinant *Escherichia coli* (Raza et al., 2018).

The most commonly studied PHA biosynthesis from different microorganisms are poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Akdoğan & Çelik, 2021). In some bacteria, the genes coding for the proteins involved in the biosynthesis of polyhydroxyalkanoates are arranged as one phaCAB operon (Sharma & Dhingra, 2021). The process of scl-PHA biosynthesis is initiated when two molecules of acetyl-CoA join with acetoacetyl Co-A with the help of an enzyme 3-ketothiolase (PhaA). Then by using an enzyme acetoacetyl-CoA reductase (PhaB) and NADPH as the electron donor acetoacetyl-CoA is reduced to 3-hydroxybutyryl-CoA, which is then polymerized to PHB by PHA synthase enzyme (PhaC class I) (Alkotaini et al., 2018). PHBV can be formed either from the addition of acetyl-CoA and propionyl-CoA by the action of 3–ketothiolase to 3-ketovaleryl-CoA and make 3HV monomer by adding propionate to the culture medium or from the polymerization of 3-ketoacyl-CoAintermediates, which are produced from the partial oxidation of substrates obtained by the β-oxidation of butyrate, valerate and long-chain fatty acids (LCFAs) (Blunt et al., 2018).

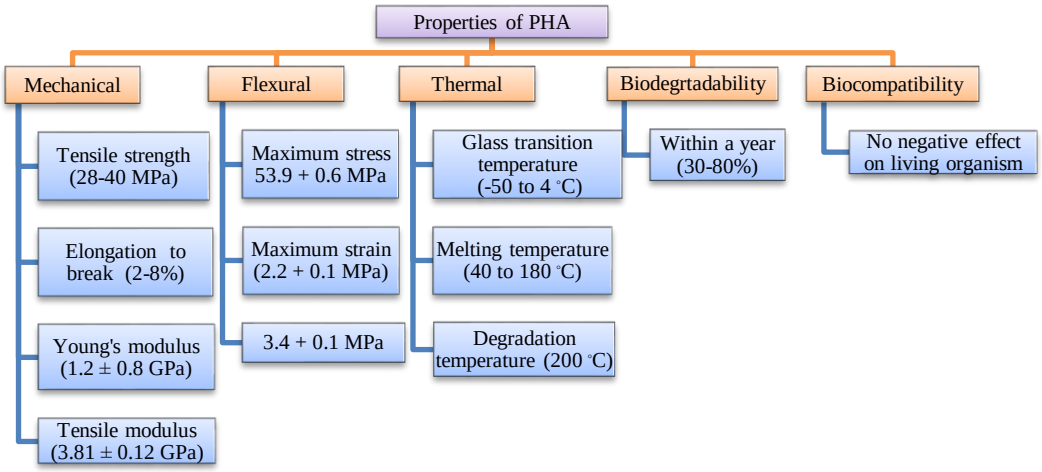

Fig. 3. Some common properties of polyhydroxyalkanoates

Some bacteria use starch, glucose, glycerol and amino acids as a carbon source to synthesize propionyl-CoA, which is further used in the biosynthesis of PHBV by producing the main precursor of 3HV-CoA. It was reported that some bacteria use a single pathway for generating propionyl-CoA for 3HV unit formation while others can use multiple pathways (Fig. 4), for example only the methyl malonyl-CoA pathway is commonly used by *N. corallina* (Koller, 2019) while *H. mediterranei* follows four alternative pathways; aspartate/2-oxobutyrate, citrate-malate/2-oxobutyrate, 3-hydroxypropionate, and the methyl malonyl-CoA pathway (Fig. 4) (Behera et al., 2022). In the aspartate and citrate-malate pathway, propionyl Co-A is synthesized from 2-oxobutyrate, started either from methionine and threonine or acetyl-CoA and pyruvate. In the third pathway, propionyl-CoA is produced from acetyl-CoA and CO_2 while in the fourth pathway, propionyl-CoA is produced from methylmalonyl-CoA, which is an isomerized product of succinyl-CoA (Qiao et al., 2023). Mcl-PHA biosynthesis occurs either *de novo* (pathway III) or by the β-oxidation of fatty acids (pathway II) and it is widely studied in *Pseudomonas putida* and *P. oleovorans* (Blunt et al., 2019). These pathways convert carbon sources into different (R)-3-hydroxyalkanoyl-CoA molecules, which are further used to synthesize PHA by the enzyme class II PHA synthases. Mcl-PHA biosynthetic genes are arranged as phaC1ZC2 and phaIF operons in which PhaD works as a transcriptional activator in the presence of β-oxidation metabolites (Andler et al., 2021). Mcl-PHA intermediates acyl-CoA, which is the end product of β-oxidation, is oxidized into enoyl-CoA, which is then converted into (S)-3-hydroxy acyl-CoA and 3-ketoacyl-CoA. It is used by (R)-specific enoyl-CoA hydratase (PhaJ) for the synthesis of (R)-3-hydroxy acyl-CoA (PhaJ). In another pathway of mcl-PHA biosynthesis, acetyl-CoA is converted into malonyl-CoA, which is then transacylated into acyl-ACP-CoA. In most of the *Pseudomonas* sp. this acyl-ACP-CoA reacts with (R)-3-hydroxy acyl-CoA for the biosynthesis of PHA by using an enzyme acyl-ACP-CoA specific transacylase (PhaG) (Yan et al., 2022).

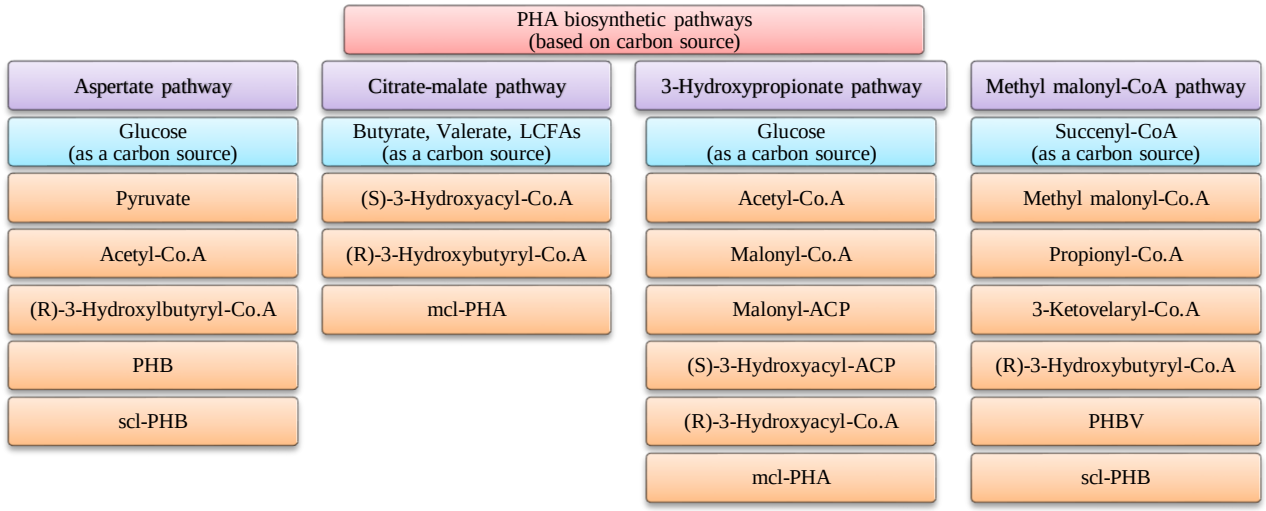

Fig. 4. Different pathways of PHA biosynthesis based on carbon source

Use of fruit wastes for PHA biosynthesis

The fruit processing and manufacturing industry produces fruit wastes throughout the entire production phase due to damage during transport, storage, processing and inappropriate packaging. The resi-

dues from fruits and vegetables are rich in simple and complex sugars, which can be utilized as a carbon source for the growth of microorganisms to produce biogas, bio-ethanol, and biopolymers (Kumar et al., 2020). Agricultural and agro-industrial processing produces huge amounts of fruit waste, which are more than half of all the weight of fresh fruit and sometimes nutritional and functional content is greater than end products. Rebocho et al. (2020) made a natural blend of scl-PHA and mcl-PHA by the co-culturing of *C. necator* and *P. citronellolis* on apple pulp extract. They were found to have 48% w/w of 3HB, 35% w/w of 3HD, 10% w/w of 3HO, 3% w/w of 3HDd, 3% w/w of 3HTd and 0.5% w/w of 3HHx. Furthermore, when grown individually it was observed that *C. necator* produces 3HB only while *P. citronellolis* synthesizes 3-hydroxytetradecanoate(3HTD), 3-hydroxydodecanoate (3HDD), 3-hydroxydecanoate (3HD), 3-hydroxyoctanoate (3HO) and 3-hydroxyhexanoate (3HHx) (Rebocho et al., 2019). Banana is a low-caste agricultural material that can be used as a carbon source for PHA synthesis as it contains 2.1% and 0.4% glucose, fructose and sucrose in the pulp and peel respectively while it contains 18.4% starch in pulp and 6.1% LCB in peel (Tripathi et al., 2022). Getachew and Woldesenbet observed that *Bacillus* sp. biosynthesizes 27% w/w PHB (2.1 g/L) using banana peel hydrolysate as a sole carbon source, which was produced by the zinc chloride method. It was lower in concentration as compared to 61% PHB (6.1g/L) when glucose was used as a carbon source (Getachew & Woldesenbet, 2016). Some common by-products produced from the fruit processing industry and the bacteria capable of utilizing it as a carbon source for the biosynthesis of PHA are listed below in Table 1.

Table 1
List of bacteria capable of utilizing fruit waste as a carbon source for the intracellular biosynthesis of PHA

Bacteria	Fruit and its wastes as a sole carbon source	Biomass concentration, g/L	PHA content, g/L	PHA content, %	Type of PHAs	References
Bacillus cereus	grape peel	2.82	0.53	18.8	PHB	Andler, Valdés et al. (2021)
Bacillus thuringiensis IAM12077	mango peel	7.86	4.03	51.3	PHB	Gowda & Shivakumar (2014a)
Recombinant *Bacillus subtilis*	orange peel	3.02	1.24	41.0	PHB	Sukan et al. (2014)
Bacillus subtilis	papaya peel	15.0	11.65	77.67	PHB	Rao et al. (2019)
	orange peel	19.39	9.68	49.93		
Bacillus cereus	pineapple	2.46	1.0	40. 54	PHB	Suwannasing et al. (2015)
Bacillus velezensis BTR2015	pomegranate peel	6.0	4.5	75.0	PHA	Rayasam et al. (2020)
Bacillus siamensis RET2912	pomegranate peel	5.50	4.0	73.0	PHA	Rayasam et al. (2020)
Bacillus subtilis PVR2988	pomegranate peel	8.0	5.75	71.0	PHA	Rayasam et al. (2020)
Bacillus halotolerans KSI1507	pomegranate peel	74.0	27.0	36.50	PHA	Rayasam et al. (2020)
Bacillus halotolerans DSM1802	pomegranate peel	7.2	6.0	83.0	PHA	Rayasam et al. (2020)
Bacillus siamensis LFS1715	pomegranate peels	2.0	1.5	75.0	PHA	(Rayasam et al. (2020)
Cupriavidus necator	pineapple peel	5.3	0.7	12.7	PHB	Sukruansuwan & Napathorn (2018a)
	pineapple core	6.1	2.1	35.6		
Cupriavidus necator	purified grape seed oil	8.3	6.4	76.8	PHB	Kovalcik et al. (2020)
	spent coffee grounds oil	10.0	6.5	65.3		
	waste fried sunflower oil	8.6	76.1	70.4		
	fructose	8.9	5.5	61.6		
	grape sugar extract	4.1	1.9	47.2		
Cupriavidus necator	pomegranate peels	4.0	2.4	60.0	PHA	Rayasam et al. (2020)
Cupriavidus necator and *Pseudomonas citronellolis*	apple pulp waste	6.93	3.03	43.7	PHB	Rebocho et al. (2020)
Cupriavidus necator B-10646	jerusalem artichoke (tubers)	66.9	–	82	P(3HB-co-3HV), P(3HB-co-4HB)	Volova et al. (2021)
	jerusalem artichoke (vegetative biomass)	55.1	–	62		
Cupriavidus necator B-10646	sugar beet molasses	80–85	65	80	PHA	
Geobacillus stearothermophillus TKR1707	pomegranate peels	8.5	6.5	76.0	PHA	Rayasam et al. (2020)
Halomonas campilasis	banana peel	0.776	0.329	13.6	PHBV	Kulkarni et al. (2015)
	orange peel	0.514	0.11	23.8		
Halorhodospira halophila	glucose	5.1	3.7	72.5	PHB	Kovalcik et al. (2020)
	grape sugar extract	3.1	1.8	57.0		
Halomonas organivorans	glucose	6.0	3.9	66.0	PHB	Kovalcik et al. (2020)
	grape sugar extract	3.9	2.1	55.4		
Haloferax mediterranei	date waste extract	18.0	4.5	25.0	PHBV	Alsafadi et al. (2020)
Klebsiella pneumoniae	watermelon	68.72	22.61	32.90	PHB	Valdez-Calderón et al. (2022)
	papaya	86.36	23.72	27.47		
	orange	88.58	23.38	26.40		
	banana peel	82.77	25.11	30.34		
Pseudomonas resinovorans	grapes	6.1	1.42	23.3	mcl-PHA	Follonier et al. (2014)
Pseudomonas putida	grapes	7.9	5.8	41.1	mcl-PHA	Follonier et al. (2015)
Staphylococcus aureus ESR1315	pomegranate peel	3.0	2.0	67.0	PHA	Rayasam et al. (2020)
Tepidimonas taiwanensis LMG 22826	grape pomace extract	4.36	2.09	50.12	PHA	Kourilova et al. (2021)
Cupriavidus necator DSM 545	tomato	1.3 ± 0.03	0.4 ± 0.1	34.6 ± 2.5	3HB and 3-hydroxybutyrate (3HV)	Costa et al. (2022)
	pear	0.8 ± 0.04	0.4 ± 0.2	54.5 ± 11.3		
	red apple (filtration)	6.2 ± 0.2	4.9 ± 0.03	79.1 ± 0.9		
	red apple (autoclaving)	6.7 ± 0.04	2.3 ± 0.1	34.5 ± 2.1		
	melon (filtration)	5.8 ± 0.4	4.3 ± 0.1	73.8 ± 10.1		
	melon (autoclaving)	4.2 ± 0.4	34.0 ± 6.7	1.43 ± 0.2		
Cupriavidus necator H16	banana frond extract	3.6	–	37.4	PHB	Low et al. (2021)
Cupriavidus necator A-04	crude aqueous extract of pineapple waste	13.6	–	60.1	PHB	Sukruansuwan & Napathorn (2018b)
Cupriavidus necator DSM 545	melon extract	5.1	4.6	74.9	PHB	Costa et al. (2023)
Cupriavidus necator DSM 545	red apple extract	10.9	–	67.9	PHB	Costa et al. (2022)
Cupriavidus necator DSM 545	melon waste (mixed microbially catabolized)	–	1.7	–	P(3HB)	Costa et al. (2023)
	acidogenic VFAS from melon waste	–	2.7	–	P(3HB-co-3HV)	

Bacteria	Fruit and its wastes as a sole carbon source	Biomass concentration, g/L	PHA content, g/L	PHA content, %	Type of PHAs	References
Pseudomonas chlororaphis subsp. *aurantiaca* DSM 19603	apple pulp	8.74 ± 0.20	–	49.3 ± 4.1	PHA	Pereira et al. (2021)
Haloferax mediterranei	date waste biomass	12.82	3.20	–	PHA	Alsafadi et al. (2020)

Furthermore, Kulkarni et al. (2015) found 22% w/w PHB of its cell dry weight when *Halomonas campisalis* was cultured on 1% v/v of banana peel extract. Naranjo et al. (2022) grew *P. sacchari* IPT101 on the banana peel and pulp for the analysis of technical, environmental and economic problems. They obtained data on fermentation conditions from the different sources and accounted for a two-step pretreatment ie; peel hydrolysis and starch hydrolysis (gelatinization, liquefaction and saccharification) for LCB and then a separation step to obtain the cells (Liguori & Faraco, 2016). The authors noticed that not only is water saving possible but global energy requirements can be reduced by up to 30.6% by the use of banana residues in different biorefineries (Liguori & Faraco, 2016).

The citrus fruit processing industries produce about 10 million metric tons of fruit waste globally, which includes more than half of the total fresh fruit mass (Zema et al., 2018). It was estimated that citrus peels contain 30% sugar (glucose, fructose, sucrose), 12% cellulose, 12% hemicellulose, 19% pectin and other phytochemicals including polyphenols-flavonoids (polymethoxylated flavones – hesperidin, naringin, nobiletin, tangeretin), essential oils (D-limonene), pigments (carotenoids), carbohydrates (pectin, cellulose, hemicellulose, and dietary fibers), flavoring compounds and pigments (Maqbool et al., 2020). Being a potent source of carbohydrates and phytochemicals, citrus peel is utilized in foods, beverages, perfumery, drugs, and the cosmetic industry. Considering only sweet lime peels, its dry weight is estimated to contain cellulose (34.8%), hemicellulose (4.52%), lignin (8.34%), and pectin (6.73%). 28.1% glucose, 2.9% galactose, 4.5% arabinose, 2.0% xylose, 1.1% mannose, and 0.6% rhamnose as simple sugars in sweet lime waste was calculated by the author using the whole fruit (John et al., 2022). Sousa et al. reported that grapes have high sugar content with glucose and fructose representing about 99% of all the sugars. They observed that 29.2% of the available sugars from the mixture of pulp, peel and seed are directly available for the fermentation (Sousa et al., 2014). Later on, Varandas et al. obtained 14.2 g in peels and 67.5 g glucose per kg of grape and reported that sugar content in grapes depends on their variety and the collecting date (Chavan et al., 2023). After pretreatment, it was noticed that grape peel contains a high amount of lignin (30% dw) while glucose is always present in higher concentrations than fructose (Basso et al., 2018).

A survey conducted by Andler et al. (2021) reported that the postharvest losses of pineapple in Assam state alone is about 9.25%. The carbohydrate content of pineapple is up to 85% of total solids whereas fiber makes up for 2–3% and it is also rich in vitamin C, calcium, potassium, iron etc. Pineapple waste produced by juice processing industries can be used as a sole carbon source for the growth of *Bacillus* sp. SV13 to obtain PHB. The maximum PHB biosynthesis was 40.54% of the cell dry weight when aeration was 2.5 vvm (Andler et al., 2021). Sukruansuwan and Napathorn pretreated pineapple residue by 1.5% v/v of sulphuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) separately to release the fermentable sugar, they obtained 0.81 g and 0.7 g sugar per gram of dry pineapple core respectively. They obtained a maximum PHB content of 12.7% and 35.6% of cell dry weight by growing *C. necator* on pineapple peel and core hydrolysate respectively (Sukruansuwan & Napathorn, 2018a). Furthermore, Vega-Castro et al. (2016) performed acid-assisted heat treatment of pineapple peel by using 2% H_2SO_4 at 121 °C and 15 psi. They cultured the bacteria at 30 °C and 200 rpm for 60 h and observed the biosynthesis of PHB at C/N ratio 11 and the C/P ratio 6. They found that in most of the conditions 3-HV was greater as compared to 3-HB (Vega-Castro et al., 2016).

In addition, Costa et al. (2022) used two different strategies for sterilization (either filtration or autoclaving) of red apple and melon and only filtration for tomato and pear. They reported that remains from red apples and melons were used as the most suitable feedstocks for PHA biosynthesis. *C. necator* DSM 545 accumulates up to 7.4 and 4.3 g/L of 3-hydroxybutyrate (3HB) from melon and red apple under particular conditions, which makes up 73.8 ± 10.1% and 67.9 ± 0.6% of its biomass, respectively. It is likely that autoclaving may provide a quick and low-cost alternative to filtering, which is an extremely costly and time-consuming process. Implementing the sterilization approach, when melon was used, autoclaving triggered 3HB accumulation and elicited 3HV production up to 0.23 g/L. This is most probably due to the release of molecules under autoclaving conditions that can act as 3HV precursors for *C. necator* DSM 545. Conversely, in the case of red apples, a lower maximum 3HB accumulation of 30.8% was observed when autoclaving was used as the sterilization technique rather than filtration (Costa et al., 2022).

Furthermore, Low et. al. analyzed the fermentation process using 40% (v/v) raw and sucrase enzyme-pretreated banana frond extract (BFE) and compared to the PHB production by *Cupriavidus necator* H16. Following enzymatic hydrolysis, the fructose level in the enzyme-pretreated BFE increased to 14.6 g/L. In comparison to the 40% (v/v) original BFE, the fermentation using the 40% (v/v) pre-treated BFE revealed increased PHB content in biomass (37.4%) and PHB concentration (1.3 g/L). The outcome shows that 40% (v/v) of BFE that has been enzyme-pretreated can be used as a substitute, sustainable, and renewable carbon feedstock to produce PHB (Low et al., 2021).

Pineapples are the third most significant tropical fruit produced globally, yielding about 24.8 million tons of fruit yearly. Based on raw materials, pineapple wastes (peel and core) are produced in significant quantities or about 59.4%. By investigating the viability of using pineapple wastes to support the high-value-added manufacturing of biodegradable polyhydroxybutyrate (PHB), Vibhavee Sukaruansuwan and Suchada Chanprateep Napathorn intended to fill the research gap. They discovered that the amount of PHB produced from the core hydrolysate was 5.88 ± 0.25 g/L of cell dry weight and 35.6 ± 0.1% (w/w) PHB content. When pineapple waste products (peel and core) were used as a culture medium, the results showed that the greatest PHB content of 60.0 ± 0.5% (w/w), the cell dry weight of 13.6 ± 0.2 g/L, the yield (YP/S) of 0.45 g PHB/g PHB substrate, and the productivity of 0.160 g/(L h) could be achieved (Sukruansuwan & Napathorn, 2018b).

The first demonstration of the feasibility of generating PHAs from fruit waste utilizing acidogenic-derived H_2 and CO_2 is provided by Costa et al. (2023). In the first stage, melon waste was continuously catabolized by a mixed microbial population to produce H_2 (26.7%) and CO_2 (49.5%), which were subsequently utilized by *C. necator* DSM 545 in a second bioreactor to produce 1.7 g/L P(3HB). Additionally, 2.7 g/L of P(3HB-co-3HV) was produced by processing the VFAs (13 g COD/L) generated during acidogenesis (Costa et al., 2023).

To synthesize medium-chain length polyhydroxyalkanoates, Pereira et al. (2021) cultured *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603 on apple pulp, a glucose- and fructose-rich waste product generated during juice manufacturing. They achieved a polymer content of 49.25 ± 4.08% and a cell dry mass of 8.74 ± 0.20 g/L. The biopolymer was a mixture of 14.5 ± 1.1 mol/L 3-hydroxybutyrate, 11.1 ± 0.6 mol/L 3-hydroxytetradecanoate, 10.1 ± 0.5 mol/L 3-hydroxydodecanoate, 3.7 ± 0.2 mol/L 3-hydroxyhexanoate, and 42.7 ± 0.1 mol/L 3-hydroxydecanoate (Pereira et al., 2021). Furthermore, Alsafadi et al. (2020) examined the possibility of using date waste (DW) biomass as a feedstock for the halophilic archaeon *Haloferax mediterranei* to manufacture PHA. In DW extract medium without trace element supplementation, a maximum cell dry mass of (CDM) (12.8 g/L) and PHA concentration of (3.20 g/L) were obtained, suggesting that DW is a suitable source for trace element (Khorshidian et al., 2022).

Methods of pretreatment

In general, the main purpose of pretreatment is to reduce the cellulose crystallinity and to increase the porosity of the lignocellulosic biomass as well as achieve partial or total delignification of the material. Some common pretreatment methods are listed below (Fig. 5).

Physical pretreatment includes milling, extrusion, freezing and microwave irradiation. By using this process, particle size of the lignocellulosic biomass can be reduced, making it more suitable for further degradation but is not more effective (Kumari & Singh, 2018).

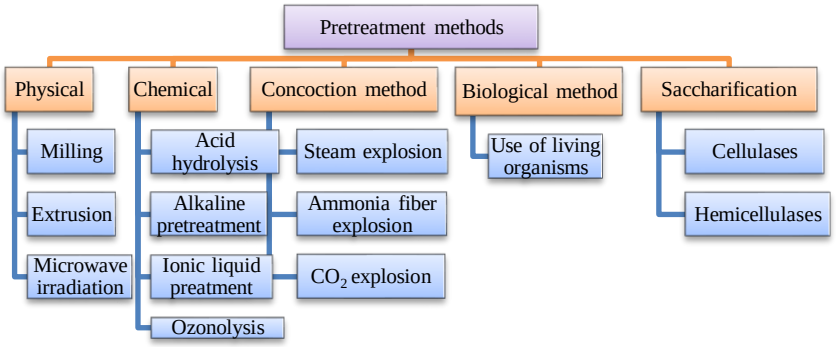

Fig. 5. Different types of methods for the pretreatment of carbon sources

Grinding is a good pretreatment but cellulose and lignin present in lignocellulosic biomass are fibrous in nature so it is more resistant to grinding (Cai et al., 2017). Because this process is unable to completely remove lignin, cellulose and hemicelluloses, it requires an additional method due to which cost relatively increases. It was observed that pretreatment before the enzymatic saccharification increased the glucose yield (Chen et al., 2017). Based on the method of feeding raw materials and removal of the final product, milling techniques can be classified into ball, hammer, two-roll, disk, and colloid milling. The critical disadvantage of this method is that overall upstream and downstream costs increase due to greater energy consumption (Boro et al., 2022). To overcome the above problem, wet disk milling can be used but the productivity is lower as compared to ball milling. Kaur et al. (2022) observed that ball milling produces a greater yield of glucose; 89.4% and xylose; 54.3% as compared to the wet disk milling yield of glucose; 78.5% and xylose; 41.5% (Kaur, 2022), whereas, compared to chipping pretreatment of Norway spruce, ball milling produces the smallest particles but has poor hydrolytic productivity (Periyasamy et al., 2022). Furthermore, it was proved that a blend of hammer and air in the ball milling process limits the power use as well as it also increases the sugar yield by enzymatic hydrolysis of Douglas fir forest products (Gu et al., 2018). The main significance of ball milling is the removal of lignin from the internal structure of reed straw by non-diffusional method (Bychkov et al., 2018). Extrusion is a process of changing physicochemical properties by the heating and use of shearing materials. It is better than milling because of high shear, short residence time, quick mixing, adaptability to process change, moderate barrel temperature, easy scale-up, and the capacity to operate continuously as well as the absence of furfural and hydroxymethyl furfural formation (Pérez-Rodríguez et al., 2018). Kuster et. al. reported that the crystallinity index of the sugarcane bagasse decreases from 57% to 54% after extrusion (Moro et al., 2017). In the microwave irradiation process, biomass absorbs microwave radiation (wavelength range 300 MHz – 300 GHz) and their molecules are excited to a higher energy level for breaking the chemical bonds (Gao et al., 2019). It destroys the silicified waxy surface, breaks the ultrastructure of cellulose as well as partially removing lignin and hemicelluloses. Imrak et al. (2018) observed that microwave pretreatment of switchgrass and *Miscanthus* increases their solubility in water for hydrogen generation by decreasing the recalcitrance of the biomasses constituents. It is eco-friendly cost-effective, consumes less power and can be carried out in the smallest area and with a shorter time duration (Kostas et al., 2017).

In the chemical pretreatment process, the lignocellulosic biomass is pretreated with dilute or concentrated acids, alkaline solutions, organic solvents, ionic liquids, and ozone gas (Selvakumar & Sivashanmugam, 2020). In the acid hydrolysis pretreatment process, either dilute or concentrated H_2SO_4, HCl or H_3PO_4 is used for pretreatment. Dilute acid pretreatment involves 0.2–2.5% of acid solution added to feedstock and mixed at 120–210 °C (Pant & Kuila, 2022) while concentrated acid pretreatment uses 65–86% of H_2SO_4, 41% of HCl or 85% H_3PO_4 at 30–60 °C temperature. However, it has some disadvantages such as the corrosiveness of instruments, creation of toxicity and synthesis of inhibitors like furan and hydroxymethylfurfural during fermentation (Hoang et al., 2021). Chiranjeevi et al. (2018) used 1% boric acid, 0.75% H_2SO_4 and 0.5% glycerol for the delignification of rice straw. Luo et al. (2022) pretreated the lignocellulosic biomass by alkaline solutions of KOH, NaOH, $Ca(OH)_2$, NH_3 and NH_4OH at a suitable temperature for a specific time. It was observed that the physical characteristics of feedstocks like surface area, porosity and crystallinity were changed while delignification occurred by de-esterification (Luo et al., 2022). Additionally, the amount of fermentable sugar was also increased by decreasing the concentration of inhibitors of saccharification. Nargotra et al. (2018) observed that 0.5% sodium hydroxide solutions can enhance the enzymatic saccharification of pretreated sunflower stalks pulp. Ionic liquids are organic salts with melting points less than 100 ^{0}C and have cations and anions (Naranjo et al., 2022). They can dissolve carbohydrates and lignin simultaneously. Biofuel with 70% conversion efficiency was produced by the use of ethanolamine acetate (Sun et al., 2017). Tolesa et al. extracted 71.2% lignin from coffee husk by the use of ammonium-based ionic liquids (Tolesa et al., 2018). The main disadvantage of this method is that it can denature the cellulase enzyme (Aggarwal et al., 2022). In the ozonolysis pretreatment process, ozone gas is used for delignification by breaking aromatic rings, as well as also partially saccharifying cellulose and hemicellulose (An et al., 2022).

In order to reduce the operational costs of different pretreatment techniques, researchers use biological processes. It involves the pretreatment of lignocellulosic feedstocks by the microorganism for the breakdown of cellulose, hemicellulose and lignin so that it can be used as a carbon source for PHA biosynthesis (Sindhu et al., 2016). Bhattacharjya et al. (2021) used a microbial consortium of cellulolytic genera of *Bacillus*, *Aspergillus*, *Candida*, and *Streptomyces* for the decomposition of organic matter. It was observed that white and soft rot fungi can degrade lignin and brown rot fungus broke down cellulose by synthesizing different enzymes likes lignin peroxidases, polyphenol oxidases, laccases and manganese-dependent peroxidases (Dev et al., 2019). Shi et al. reported 35.5% delignification by solid-state cultivation of *P. chrysosporium* on cotton stalk. The authors also examined the pretreatment efficiency of *Ceriporiopsis subvermispora* for ethanol production from corn stover and observed 31.6% delignification by preserving 94.0% of cellulose (Kumari & Singh, 2018).

Some other concoction pretreatment methods are reported to release the soluble sugars from lignocellulosic biomass, for example steam explosion pretreatment, ammonia fiber explosion pretreatment, CO_2 explosion pretreatment and saccharification. Steam explosion pretreatment involves the pretreatment of ground biomass by high-pressure saturated steam, following which the pressure is quickly removed due to which explosive delignification occurs (Bonfiglio et al., 2019). It includes a pressure of 20–50 bar and a temperature of 160–270 °C (Ouyang et al., 2018). Ilanidis et al. (2021) observed that at higher temperatures sometimes fermentation inhibitors are synthesi-

zed which can reduce 20–25% of saccharification yield by removing the soluble sugars. In the ammonia fiber explosion pretreatment process, the lignocellulosic biomass is pretreated with liquid ammonia under high pressure of about 1.72–2.06 MPa and heated to an optimum temperature of 60–120 °C for about 30 min. After maximum temperature is attained, the rapid expansion of ammonia gas occurs by the release of pressure due to which lignin degrades and improves biomass digestion (Zhao et al., 2020). It was reported that the suitable condition for the pretreatment of maize stover is ammonia to biomass loading ratio (1:1) at the temperature of 90 °C with 60% moisture (Pandey et al., 2019). Furthermore, Joy & Krishnan (2022) observed that the best conditions for converting sweet sorghum bagasse to ethanol are ammonia to feedstock ratio of 2:1 at 140 °C for 5 min with 120% moisture content. At high pressure, supercritical carbon dioxide (SC-CO_2) enters the feedstock and creates carbonic acid by dissolving in water, which increases the hydrolysis of lignocellulosic biomass and the availability of fermentable sugars (Dharmaraja et al., 2023).

Advancements in cellulolytic and hemicellulolytic enzymes have significantly enhanced enzymatic processing, reduced costs and improved efficiency in various biotechnological applications (Østby et al., 2020). Cellulolytic enzymes, such as cellobiohydrolases (GH6 and GH7), endo-β-1,4-glucanase, and β-glucosidase, play a crucial role in breaking down cellulose (Fig. 6). GH6 cleaves cellobiose from the non-reducing end, while GH7 targets the reducing end of cellulose chains. Endo-β-1,4-glucanase cleaves β-(1,4)-linkages in non-crystalline cellulose regions (Soni et al., 2023), and β-glucosidase releases D-glucose from the non-reducing end of oligosaccharides. Hemicellulolytic enzymes, including xyloglucanase, endo-β-1,4-xylanase, endo-β-1,4-mannanase, β-xylosidase, and β-mannosidase, target hemicellulose by cleaving specific linkages and releasing monosaccharides like D-xylose and D-mannose. β-(1→4)-links in xyloglucan chains, a significant hemicellulose component, are broken down by xyloglucanase (Yuan et al., 2001). Endo-β-1,4-xylanase helps hydrolyze one of the most prevalent forms of hemicellulose by targeting β-(1→4)-linkages in xylan chains (Soni et al., 2023). Similarly, β-(1→4)-linkages in glucomannan main chains, another important hemicellulose polymer, are broken by endo-β-1,4-mannanase. β-mannosidase eliminates D-mannose residues from the non-reducing ends of glucomanno-oligosaccharides, whereas β-xylosidase cleaves unsubstituted D-xylose from the non-reducing ends of xylo-oligosaccharides (Østby et al., 2020). Additionally, hemicelluloses' debranching enzymes are specialised enzymes that help break down hemicellulose by removing side-chain substitutions. These include α-galactosidase, which eliminates α-(1→6)-linked D-galactosyl substitutions from glucomannan and glucomanno-oligosaccharides, and α-arabinofuranosidase, which cleaves L-arabinosyl substitutions from xylans and xylo-oligosaccharides (Østby et al., 2020). Acetyl groups at different locations in xylans, xylo-oligosaccharides, glucomannans, and glucomanno-oligosaccharides are hydrolysed by α-glucuronidase. While glucuronoyl esterase targets ester bonds between lignin alcohols and 4-O-methyl-D-glucuronic acid substitutions on the xylan backbone, feruloyl esterase cleaves hydroxycinnamoyl groups esterified to arabinosyl substitutions in xylan or lignin. By exposing the primary polysaccharide chains for further enzymatic activity, these enzymes significantly improve the breakdown of hemicellulose and support biomass conversion processes (Mnich et al., 2020). Lytic polysaccharide monooxygenase (LPMO) complements these processes by oxidatively cleaving cellulose chains at the C1 carbon. Collectively, these enzymes enable the efficient breakdown of plant cell wall components, unlocking fermentable sugars essential for biofuel production and other industrial applications (Yu et al., 2023).

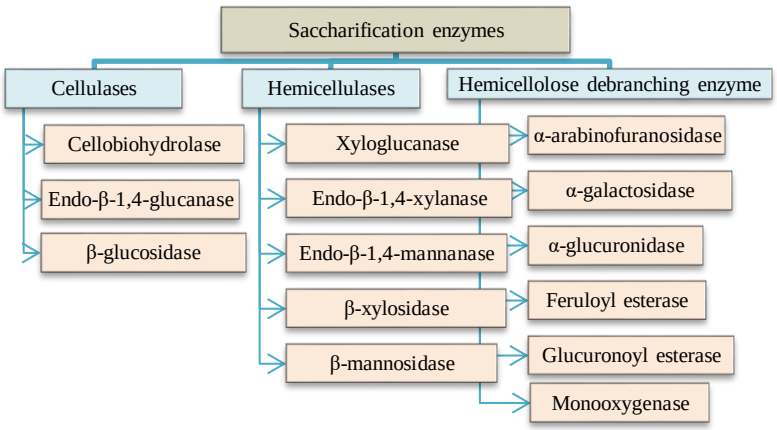

Fig. 6. Different types of enzymes involved in the saccharification process

Biodegradation of PHA and PHA-based composites

Natural degradation of PHA by the cell-mediated process is known as biodegradation. It takes a few days to a few months depending on the environment ie; soil, activated sludge, marine or freshwater etc. (Meereboer et al., 2020). Chen et al. (2023) observed that the complete PHA was degraded in phosphate buffer, muscular tissue of mammals, human blood and serum. Furthermore, it was reported that biodegradation of biopolymer by the microorganisms occurs at the amorphous end rather than the crystalline region through the enzyme-mediated process to produce monomers and oligomers, which are further metabolized under aerobic conditions to give water and carbon dioxide whereas under anaerobic conditions methane was obtained. Several factors can affect the enzymatic hydrolysis of PHAs, such as PHA processing techniques (extrusion, pellets, solvent cast film, electrospinning), physiochemical properties of PHAs (monomer composition and distribution, geometry, end group, crystal size, lamellar thickness and glass transition temperature), soil condition (soil type, microbial population, humidity and pH) and weather condition (temperature). It has been revealed that PHAs with a shorter side chain and homopolymer show a lower rate of degradation than with a longer side chain and copolymer.

Moreover, biodegradation of PHA can be determined by physical methods (physical appearance, mass reduction and strength properties), reactive pyrolysis gas chromatography (change in chemical composition ratio) (Baidurah et al., 2019) and respirometric methods (biochemical oxygen demand, gas evolution) (Dilkes-Hoffman et al., 2019).

Biodegradation mechanisms. The process of biodegradation starts with the colonization of bacteria that secrete PHA depolymerase enzyme on the rough and porous surface of the biopolymer and then hydrolysis of the polyester chain occurs (Pramanik, 2023). The enzyme may be intracellular or extracellular. Intracellular PHAs' depolymerase is synthesized by the PHA-accumulating cells and hydrolyzes or mobilizes their own native PHA granules (Boey et al., 2021) whereas extracellular PHA depolymerase is produced by PHA-accumulating cells after death and cell lysis and depolymerizes extracellular polymers. PHA oligomers produced after hydrolysis are depolymerized by hydrolases and produce organic acids which are used by the microorganisms (Kalia et al., 2023).

Biodegradation of PHAs in bioreactors. Weng et al. analyzed the biodegradation of PHBV films in different conditions and they observed that 81% were degraded on a lab scale while completely degraded in the pilot-scale composting test (Salomez et al., 2019). Further, researchers examined the effect of chemical structure on the biodegra-

dation of PHB, PHBV (with 3%, 20%,40% HV) and P (3HB-4HB) (with 10% 4HB) and reported that they were degraded within 6 months under controlled composting conditions (Mouhoubi et al., 2022). It was observed that a mixture of PHB with polybutylene adipate terephthalate (PBAT) content shows selective biodegradation (Zytner et al., 2023).

Biodegradation of PHA plastic films in soil. Morse and co-worker buried P(HB-co-10%HHx) film in microcosm soil and observed that 80 % of the biopolymer was degraded within 7 days under anaerobic digester biosolids. Kim et al. used different soil to check the effect of soil type on the biodegradation of PHA and they reported that 98.9% and 7.1% of PHB was degraded in activated sludge soil and forest soil respectively within 25 days only (Boey et al., 2021).

Biodegradation of PHB and P(HB-co-HHx). The biodegradation rate of PHB increases with the addition of 3HHx monomer because it disturbs the crystal lattice and increases the amorphous region in PHB (Volova et al., 2021). Furthermore, Baidurah et al. (2019) reported that 3HHx moieties help in the degradation of 3HB with the increase in soil burial time. Moreover, Wang et al. observed that in the nutrient-depleted activated sludge P(HB-co-12%HHx) degraded by 40% as compared to 20% degradation of PHB (Baidurah et al., 2019).

Current challenges for PHA commercialization

Current challenges in PHA biosynthesis include optimizing microbial strains for enhanced PHA production, improving substrate utilization efficiency, and developing cost-effective fermentation processes. Future challenges involve scaling up PHA production for commercial viability, addressing environmental concerns related to raw material sourcing, and exploring advanced genetic engineering techniques to tailor PHA properties. Additionally, market competitiveness and regulatory frameworks will influence the widespread adoption of PHAs as sustainable biopolymers. All these challenges are broadly divided into three categories viz; biological, technological and economical challenges (Fig. 7).

The production of PHA from fruit wastes poses several biological challenges that researchers are actively addressing. One significant challenge arises during the pretreatment of lignocellulosic biomass at higher temperatures, where inhibitory byproducts such as phenolic compounds (e.g., vanillin, ferulic acid), furan derivatives (e.g., furfural, 5-hydroxymethylfurfural), and aliphatic acids (e.g., acetic acid, formic acid) are generated. These inhibitory byproducts can interfere with microbial growth at varying compositions of the fermentation medium, complicating the PHA production process (Zhai et al., 2022). Another challenge lies in the difficult genetic manipulation of PHA producers. Researchers aim to achieve high yields of pure and toxin-free PHA from bacteria, necessitating the investigation and selection of recombinant gram-positive bacteria. However, this pursuit increases the complexity of downstream processing, leading to elevated costs in PHA biosynthesis (Neifar et al., 2018). Furthermore, the limited ability of bacteria to utilize various carbon sources adds to the challenges. Only a handful of microorganisms have been discovered that can efficiently use lignin-derived monomers as the sole carbon source for PHA biosynthesis. Therefore, there is a pressing need to explore and identify new microbes with the capability to biosynthesize PHA efficiently from lignin (Kumar et al., 2017).

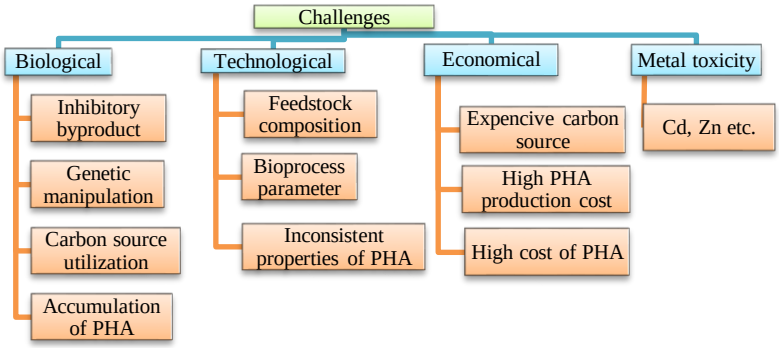

Fig. 7. Current and future challenges for the biosynthesis of polyhydroxyalkanoates (PHA) at commercial scale

In addition to these challenges, the undesirable properties of accumulated PHA are a concern. To address this, researchers recommend incorporating biobased polymers such as poly (lactic acid), thermoplastic starch, and poly (butylene adipate terephthalate) as blending agents. This strategy aims to enhance the biodegradability of PHA, making the biopolymer easily degradable (90% in 180 days) and biocompostable (with less than 10% polymer remaining in a sieve of 2 mm pore size after 180 days). By addressing these biological challenges, researchers strive to advance the sustainable and cost-effective production of PHA from lignocellulosic biomass (Koh et al., 2018).

In the realm of biotechnological advancements, the utilization of waste feedstocks for the production of polyhydroxyalkanoates (PHAs) poses several technological challenges. One significant obstacle lies in the inconsistent chemical composition of the waste feedstock, where cellulose constitutes 25–50% of total lignocellulosic dry matter, hemicellulose represents 20–40%, and lignin makes up 15–25% of the entire content. The successful production of PHAs in high concentration demands precise control over various bioprocess parameters. Additionally, downstream processing and PHA recovery present technological hurdles that must be overcome for efficient and cost-effective production. Another complication arises from the inconsistent properties of PHAs themselves, prompting the need for standardized approaches in their synthesis. Addressing these challenges, genetically modified bacteria have emerged as a promising solution. These engineered microorganisms can accumulate more PHA in their cellular biomass and exhibit the ability to produce single selected monomers, offering a more controlled and tailored approach to PHA production compared to the conventional mix of copolymers. As researchers delve deeper into these technological intricacies, advancements in waste-to-PHA processes hold significant promise for sustainable and environmentally friendly bioplastic production (Freitas et al., 2021).

The economic viability of polyhydroxyalkanoates (PHAs) as a sustainable alternative to petroleum-based plastics faces significant economic challenges. A major hindrance to large-scale production lies in the high cost associated with the carbon feedstock used for PHA synthesis (Kourmentza et al., 2017). Research efforts have been dedicated to reducing production costs, with a notable contributor being the use of high-purity substrates, constituting a substantial portion of the total production expenses (Meereboer et al., 2020). To compete with traditional plastics, it is imperative to lower overall PHA production costs by exploring more economical feedstocks. Scientific endeavors have focused on harnessing agro-industrial waste streams for fermentation processes, presenting an opportunity to make PHA economically feasible (Riedel & Brigham, 2020). Several bacterial species have demonstrated the ability to produce PHAs using waste from the agri-food industry. Another economic hurdle arises in the commercial availability of PHAs, priced at 2–10 US$/kg, making them approximately 6–10 times more expensive than petrochemically derived plastics (Khatami et al., 2021). Addressing this challenge involves seeking new microbial strains capable of converting lignocellulosic biomass into PHAs in a single step, combining saccharification and fermentation without the need for additional pretreatment steps. This innovative approach holds the potential to signif-

icantly reduce the overall cost of the final bioproduct, fostering the widespread adoption of PHAs in the quest for more sustainable plastic alternatives (Govil et al., 2020).

Govil et al. (2020) studied different groups of organic wastes including municipal solid waste combined with sludge and fruit waste and reported the migration of heavy metals (Cd, Zn etc.) from these wastes to the PHA structure. They reported that the level of contamination depends on the types of feedstocks and stabilization-extraction process. The researchers also found that PHA biosynthesized from fruit waste contains a lower concentration of heavy metals than PHA produced from municipal waste and sludge wastewater (Govil et al., 2020). It is already known that heavy metals can cause severe damage to our body. Indeed, on repeated exposure it can cause serious diseases like gastric cancer, thyroid cancer (Vigneri et al., 2017) etc.

Conclusions

There is a desire for alternatives that come from renewable resources and are biodegradable as people become more aware of the dangers of using nonrenewable materials. One of the most interesting materials that meets this requirement is biopolymer. Several approaches for the synthesis of biopolymer from fruit waste as an alternative to petrochemically derived non-renewable plastic have been discussed. Using bacteria as a major source, fruit waste may be converted into biopolymers, which are organic and biodegradable substitutes for synthetic plastics. Fruit variety, bacterial strain, and fermentation conditions are some of the variables that affect the quality of the biopolymers. Using biopolymer lowers emissions of plastic, greenhouse gases, and garbage. Cost-effectiveness, competitiveness with conventional plastic manufacture, and scaling up production for industrial applications are all obstacles, nevertheless. Although biopolymers can be employed in many sectors, safety assessments and regulatory restrictions may well be required. The economic feasibility of PHA biosynthesis is dependent on some variables, including market demand, the cost of collecting fruit waste, and the efficiency of the fermentation process. Notwithstanding these obstacles, further investigation and creativity may result in novel biopolymer compositions and enhanced uses. The manufacturing of biopolymers may be advanced by cooperation between academic institutions, the agriculture industry, and the polymer business. Raising consumer knowledge of the advantages of biopolymers has the potential to affect consumer purchasing decisions and expand the market for these goods. In conclusion, the bacterial synthesis of biopolymers from fruit waste is a viable and sustainable strategy with the potential to solve issues with waste management and the environment. It has different strengths and weaknesses but the best approach depends upon the particular biopolymer and microbe of interest. Biologically synthesized polymer has many advantages as it is eco-friendly and easily biodegradable in nature. Although very useful, its biosynthesis at a commercial scale is very difficult because of its high bioprocessing cost. Moreover, for effective implementation on a larger scale, it also presents technological, economic, and regulatory problems that must be properly analyzed and handled. Therefore, more research is still needed in this field for the scale-up of biopolymer synthesis.

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