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Review

Production of polyhydroxyalkanoates as a feasible alternative for an integrated multiproduct lignocellulosic biorefinery

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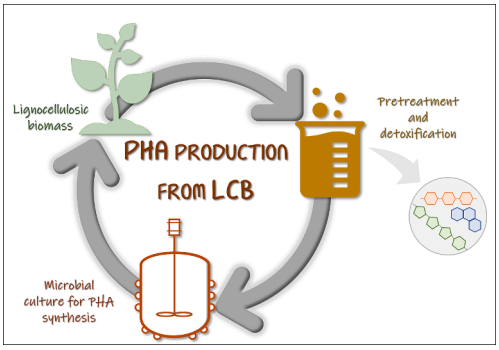
HIGHLIGHTS

- PHAs are biodegradable, biocompatible, non-toxic and thermoplastic.
- High PHA production costs are the main constraint for its industrialization.
- Lignocellulosic biomass is a renewable and cheaper feedstock for PHA synthesis.
- PHA from lignocellulosic biomass is a feasible and sustainable alternative.
- Full integration of PHA into a lignocellulosic biorefinery is getting closer.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyhydroxyalkanoates (PHAs) are considered an alternative to fossil fuel-based plastics. However, in spite of their interesting properties and their multiple applications, PHAs have not taken off as an industrial development. The reason is mainly due to the associated high-production costs, which represent a significant constraint. In recent years, the interest in lignocellulosic biomass (LCB) derived from crop, forestry or municipal waste by-products has been growing, since LCB is plentiful, cheap, renewable and sustainable. On this matter, the valorization of LCB into PHAs represents a promising route within circular economy strategies. However, much effort still needs to be made to improve the bioconversion yields and to enhance PHA production efficiency. So, this review focuses on reviewing the different options for PHA synthesis from LCB, stressing the progress in biomass deconstruction, enzymatic hydrolysis and microbial conversion. In addition, some of the current biological strategies for improving the process of bioconversion are discussed.

1. Introduction

As of today, nobody could imagine daily life without plastics. Their special characteristics, such as the low cost, durability or chemical resistance, along with other factors concerning economics and population growth, have led to an unstoppable upward trend in the global production of plastics (Patti and Acierno, 2022). However, their widespread use is being questioned by Governments and citizens due to their

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harmful consequences not only on the environment, but also on human and animal health. The problems triggered by plastics stem from their petroleum-based origin, which fosters environmental pollution and allows plastics to remain unchanged for up to hundreds of years (Yukesh Kannah et al., 2022). The lack of proper logistics at the end of the life cycle of plastics promotes their accumulation in nature, which is particularly damaging in the oceans, where the ubiquitous presence of microplastics seriously affects the marine ecosystem (Geyer et al., 2017; Zhang et al., 2022). Moreover, plastic production processes and the incineration of plastics at the end of their life discharge millions of tons of CO_2 into the atmosphere, contributing to global warming (Nicholson et al., 2021).

The EU Commission's concern about environmental degradation resulted in the European Green Deal (2020), which aims to reduce greenhouse gas emissions by 2050. Under the Green Deal, several actions have been included, such as the new circular economy action plan, which proposes a specific strategy for plastics that seeks a sustainable use of plastics and new alternative polymers that respect the environment. In order to achieve these sustainable goals, it is necessary to start looking for new polymers, and biopolymers are, in particular, the ones that are receiving more attention as possible substitutes for conventional plastics. This is due to their similar properties, as well as their sustainability, their environmentally friendly production and their numerous applications (Ibrahim et al., 2019).

Biopolymers are considered bio-based polymeric materials that are produced from natural sources, such as biomass, or from living organisms, such as plants or microbes (Christian, 2016; Yukesh Kannah et al., 2022). Overall, biopolymers are environmentally friendly; however, it must be kept in mind that a biopolymer is not necessarily biodegradable, as not all of them are capable of degradation by the action of microbial enzymatic systems (Christian, 2016). Several classifications can be considered in relation to the monomeric unit (such as proteins or polynucleotides), the type of polymer (for example, starch-based, or cellulose-based) or the origin (Yukesh Kannah et al., 2022). In terms of origin, three types of biopolymers can be distinguished: natural, synthetic and microbial (Baranwal et al., 2022). The latter is the focus of this review, specifically those called polyhydroxyalkanoates (PHAs). PHAs are considered a potential solution to replace traditional plastics, since they are biodegradable and biocompatible, with no health risks for animals or humans.

2. PHAs as biodegradable biopolymers

PHAs are naturally-occurring microbial polyesters composed of hydroxyalkanoate monomers. The linkage between the monomers is achieved by an ester bond between the hydroxyl group and the carboxyl group of another monomeric unit. Biodegradability, biocompatibility, non-toxicity or even thermoplasticity, are attractive properties which PHAs possess and that have positioned them as the trendy materials to become alternatives to petroleum-based plastics. PHAs could be classified into three groups, according to the carbon atoms in the monomeric unit: i) short chain-length PHAs (*scl*-PHA) with 3–5 carbon monomers,

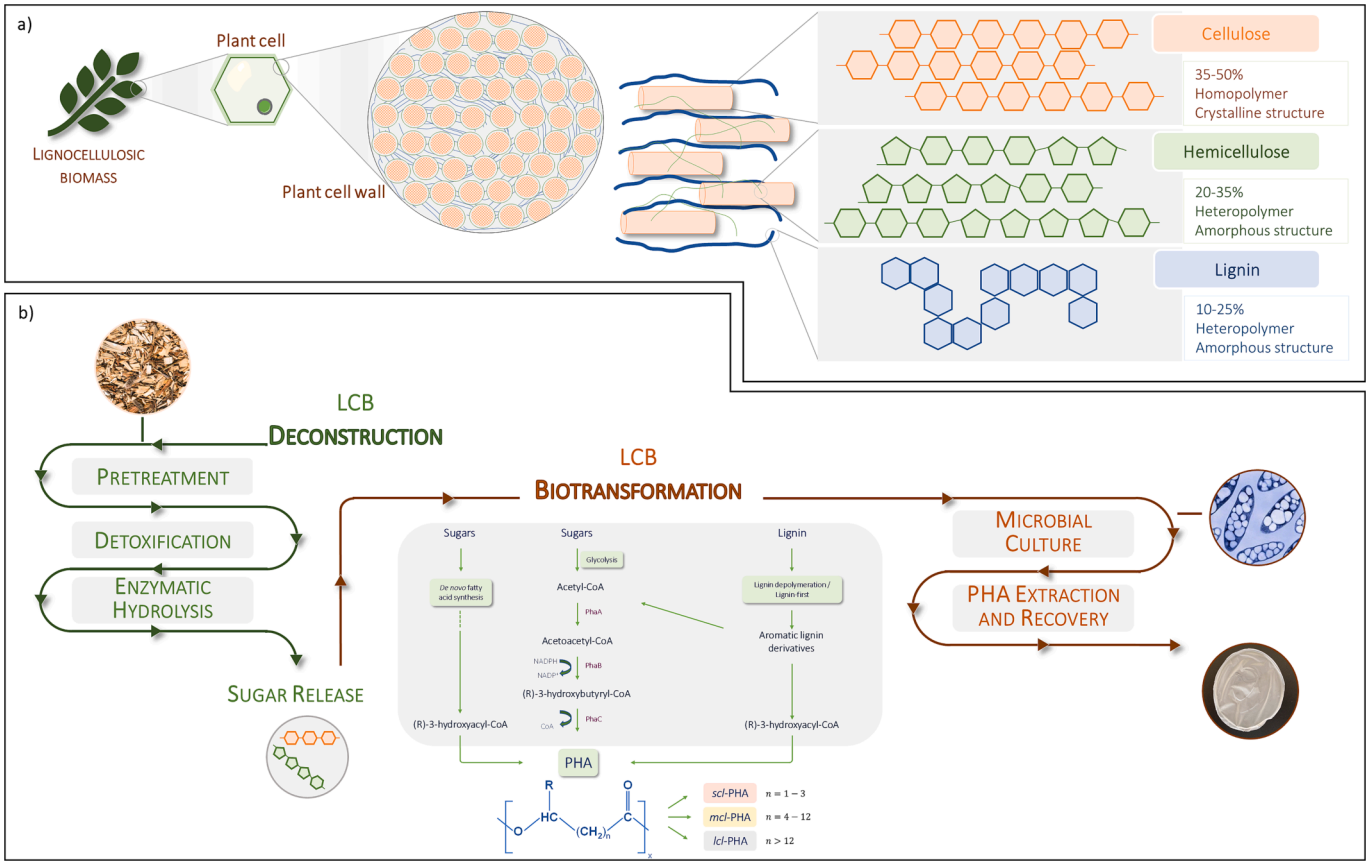

Fig. 1. a) Schematic structure of lignocellulose in plant cell wall. Lignocellulose is composed of **cellulose** (linear C6 polysaccharide that consists of D-glucose monomers linked by β-(1,4)-glycosidic bonds), **hemicellulose** (branched heteropolysaccharide of C6 and C5 units, linked by β-(1,4)- and β-(1,3)-glycosidic linkages) and **lignin** (polymer composed of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol). **b) Summary of the main steps of LCB bioconversion into PHAs**. Lignocellulosic biomass (LCB) requires a pretreatment step followed by detoxification to remove inhibitors that could affect microbial growth and enzymatic hydrolysis. Sugars and lignin released during LCB deconstruction could be metabolized into PHAs. Key enzymes are shown in their respective steps: PhaA (3-ketothiolase), PhaB (acetoacetyl-CoA reductase) and PhaC (PHA synthase). In addition, the general chemical structure of PHAs is shown, as well as the PHA classification according to the number of carbon atoms (R = alkyl group). *scl*-PHA: short chain-length PHA; *mcl*-PHA: medium chain-length PHA; *lcl*-PHA: long chain-length PHA.

ii) medium chain-length PHAs (*mcl*-PHA) with 6–14 carbon atoms, and iii) long chain-length PHAs (*lcl*-PHA), a less common polymer, made up of monomers with more than 14 carbon atoms (Arikawa and Matsumoto, 2016; Licciardello et al., 2019; Li et al., 2016) (Fig. 1). Typical examples of *scl*-PHAs are poly(3-hydroxybutyrate) (PHB) or poly(3-hydroxyvalerate) (PHV) as homopolymers, and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) as heteropolymers. In contrast, poly(3-hydroxyoctanoate) (PHO) or poly(3-hydroxynonanoate) (PHN) are common cases of *mcl*-PHAs.

Although there are exceptions, *scl*-PHAs are considered crystalline polymers with a rigid and brittle structure; whereas, *mcl*-PHAs, with a lower degree of crystallinity, are characterized by their amorphous and flexible structure and their adhesive properties (Alvarez-Santullano et al., 2021; Licciardello et al., 2019). These particular natural-based and biodegradable bioplastics are synthesized by a large group of bacteria and some members of Archaea (Zhou et al., 2023) as water-insoluble intracellular granules under high carbon availability and nitrogen or phosphorus limitation (Alvarez-Santullano et al., 2021; Li et al., 2016). In this way, PHAs represent an intracellular energy reservoir in the cytoplasm of the cell to overcome environmental stresses. A considerable list of bacterial PHA producers could be detailed, including *Cupriavidus* (formerly known as *Ralstonia*), *Bacillus*, *Pseudomonas*, *Rhodobacter*, *Halomonas*, *Azotobacter*, etc. These microorganisms are capable of metabolizing various substrates such as carbohydrates (glucose, fructose, maltose), organic acids (acetic acid, butyric acid, valeric acid), alcohols (methanol, glycerol), or even gases (methane, carbon dioxide) into PHAs (Zhou et al., 2023).

The biosynthetic pathways for PHA production vary depending on the carbon source and bacterial strain. The most widely accepted PHA biosynthesis has been described in *Cupriavidus necator* for *scl*-PHA production and is related to the central metabolic pathways, such as the pentose phosphate pathway, the Krebs cycle, or fatty acid biosynthesis that all convert the carbon source into hydroxyacyl-CoA precursors (Sagong et al., 2018). PHB is the most widely studied PHA polymer and its biosynthetic pathway is the most representative. In this route, acetyl-CoA is a key precursor for the biosynthesis of PHB, acting as a substrate for the first major enzyme involved in PHB production. Thus, acetyl-CoA acetyltransferase (3-ketothiolase: PhaA) catalyzes the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA, which is modified to (R)-3-hydroxybutyryl-CoA by NADPH-dependent acetoacetyl-CoA reductase (PhaB). Finally, PHA synthase (PhaC) polymerizes (R)-3-hydroxybutyryl-CoA monomers into a PHB growing chain, which is accumulated intracellularly at high levels, up to 80% of bacterial dry cell weight, as described in *Cupriavidus necator* (Alvarez-Santullano et al., 2021; Arikawa and Matsumoto, 2016; Możejko-Ciesielska and Kiewisz, 2016; Sagong et al., 2018) (Fig. 1).

The first report describing PHB was made in 1925 by Lemoigne in *Bacillus megaterium* (Lemoigne, 1925). However, since the 1980s, the number of reported PHAs has increased, with nearly 150 different monomers of PHA being identified (Sehgal and Gupta, 2020). Although *scl*-PHAs have been known for more than fifty years, they have not yet reached full commercial viability. Worldwide, there are slightly more than 20 companies focused on PHA commercialization, most located in Asia and the U.S., as recently reviewed in detail by Koller and Mukherjee, 2022. In Europe, there are currently only three companies focused on the commercialization of PHB and its copolymers, both at pilot and industrial scale. In Germany, the company Biomers produces PHB under the trade name of Biomer® biopolyesters, increasing its production capacity from 10 tons/year in 2007 (Alves et al., 2017) to 900 tons/year in 2022 (Koller and Mukherjee, 2022); in Italy, the company Bio-on has developed minerv®, the trade name for the generic production of PHAs that comprises more than 100 different monomers, and in the Czech Republic, where Nafigate Corporation produces PHB. However, many industries have ceased their activities in this area for various reasons, but the high cost of traditional PHA production is a common denominator. Considering that the market price of petroleum-based plastics is around US$1.2/kg, it is really hard for PHAs to compete with them as they have production prices around US$2.4–5.5/kg (Crutchik et al., 2020). Traditional methods for PHA production involve expensive carbon sources (such as pure sugars, lipids, high quality oils, etc.), pure culture, and axenic conditions, which account for 30–50% of the total production cost (Crutchik et al., 2020).

In order to tackle the growing demand for biodegradable materials, as citizens and governments are requiring, new strategies for the sustainable and competitive production of PHA are imperative. To achieve a feasible industrial process, new methods could be introduced in several ways, such as the use of cheaper carbon sources, the use of biotechnology to improve microbial strains with high production rates, or even the development of new approaches to PHA extraction and recovery using affordable and less harmful solvents. This review spotlights the use of low-cost substrates, especially those based on lignocellulosic material, as feedstock for the production of PHAs. Several recent reviews have shown the valorization of this raw material into PHAs (Dietrich et al., 2019; Obruca et al., 2015; Sohn et al., 2022), focusing on the different strategies to accomplish biopolymer synthesis and the detoxification methods to avoid microbial growth inhibition. Here, the present review discusses the ongoing development with potential applications or possibilities for integration into lignocellulosic biorefineries, with special emphasis on non-traditional and sustainable pretreatments and biological improvements that could enhance PHA production from lignocellulose.

3. Facing industrial scale PHA production limitations

3.1. Lignocellulosic biomass as a promising feedstock for PHA production

The current growing interest in the use of lignocellulosic biomass (LCB) as a carbon source for PHA production is clear. Most of the recent publications are related to the valorization of this particular feedstock, using the latest innovative technologies to reduce the cost of production of PHA. This approach fits perfectly with the European goals of zero plastic waste and also the zero carbon bioeconomy, as it offers a use for waste lignocellulosic feedstock and an alternative to petroleum-based plastics, thus promoting a circular economy. LCB represents the perfect feedstock for a sustainable production of PHAs, as it is a cheap carbon source, renewable, and globally abundant, being the most plentiful organic material, with an estimated global amount of 180–200 billion tons per year (Andhalkar et al., 2022; Vigneswari et al., 2021). In addition, this promising residue allows PHA production without competing with the human food chain, thus improving the sustainability of the global process (Abu-Thabit et al., 2022).

LCB can be sorted into three categories according to its origin as forest residues (sawdust, wood chips, dead branches, prunings), crop by-products (wheat, sugarcane, rice), and municipal solid waste (food and paper waste) (Abu-Thabit et al., 2022; Al-Battashi et al., 2019). Lignocellulose defines the plant cell wall and is responsible for providing rigidity and strength. It is composed of two types of carbohydrate polymers, cellulose and hemicellulose, and an aromatic-rich polymer, lignin. Different polymeric fractions can be distinguished in LCB, the composition of which varies greatly depending on the source, especially the plant type (Al-Battashi et al., 2019). Thus, LCB contains 35–50% cellulose, 20–35% hemicellulose and 10–25% lignin (Abu-Thabit et al., 2022; Al-Battashi et al., 2019) (Fig. 1).

Cellulose is a homopolymer and consists of D-glucose units linked by β-(1–4) linkages, which boost the repetition of cellobiose disaccharides, which in turn promote the common crystalline structure of cellulose (Abu-Thabit et al., 2022; Sohn et al., 2022). Cellulose long chains are linked by intermolecular hydrogen bonds and van der Waals forces, configuring microfibrils with a high resistance to biodegradation. In contrast, hemicellulose is a branched heteropolymer with an amorphous structure composed of carbohydrate-type monomers, as many pentoses (D-xylose, L-arabinose) as hexoses (D-mannose, D-glucose, D-galactose),

or even acetylated sugars. Xylose is widespread among crop residues and hardwoods, whereas mannose is the major sugar in softwoods (Abu-Thabit et al., 2022). The non-crystalline and amorphous structure of hemicellulose is due to the presence of side chains that configure a branched conformation (Sidana and Yadav, 2022). Lignin is a heterogenous and complex polymer with an amorphous structure, composed of three cross-linked primary monolignols (oxidatively coupled), which are *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Abu-Thabit et al., 2022; Sohn et al., 2022). These monolignols moieties are associated in lignin in the form of phenyl propanoids, called *p*-hydroxyphenyl, guaicayl and syringyl units, respectively (Kumar et al., 2022). Lignin supports the resistance and rigidity of the plant cell structure by holding together the hemicellulose and cellulose fibers. These individual polymers (cellulose, hemicellulose and lignin) do not occur in nature alone, but are bound together by chemical bonds between lignin and sugars, defining the so-called lignin-carbohydrate complexes (LCCs) (Fig. 1).

This intricate structure of LCB makes these residues very resistant to degradation, and consequently a deconstruction step is necessary to use this feedstock as a raw material for PHA production in bioconversion processes (Fig. 1). With proper pretreatment methods, the total reducing sugar yield from LCB, the carbon source for microbial bioconversion to PHAs, could be up to 90% after enzymatic hydrolysis (Xing et al., 2012). In particular, the recalcitrant nature of lignin is a hindrance to the bioconversion of lignocellulose, as it hinders the access of cellulase to specific binding sites in cellulose, thus reducing its enzymatic activity. Therefore, in order to effectively use this by-product, a pretreatment for the delignification and depolymerization is mandatory prior to performing the sugar conversion into PHAs. In general, a biorefinery seeks to transform biomass in a sustainable manner and provide a complete portfolio of high-value products, biofuels and/or energy (Andhalkar et al., 2022). To achieve these goals, an LCB-based biorefinery should combine different biotransformation methods to utilize each lignocellulosic fraction. Consequently, a lignocellulosic biorefinery requires adequate yields and the valorization of cellulose, hemicellulose and lignin. The balance between the cost of pretreatment and the yield of substrate conversion into high value-added products will determine the feasibility of the PHA production.

3.2. Pretreatment and hydrolysis alternatives for valorization of LCB into PHAs

The pretreatment of LCB is time and energy consuming and entails a high cost, approximately 40% of the total processing cost, which is the Achilles heel in the industrial scale production of PHA from lignocellulose biomass (Kumar et al., 2022; Sidana and Yadav, 2022). This outrageous cost is directly responsible for the delay in the establishment of LCB biorefineries. For about thirty years, a large number of pretreatment developments have been studied; however, various negative aspects have prevented their feasible application. This first step in the valorization of LCB is essential for the subsequent enzymatic hydrolysis and for bioconversion into PHAs. Pretreatment promotes the deconstruction of the intricate structure of LCC into intermediates, mainly sugars and aromatic derivatives, by breaking intra- and intermolecular bonds between lignin, hemicellulose and cellulose. The principal goal is to separate the main three components for individual use as carbon sources in the synthesis of high value-added products. This preceding step promotes changes in size (increasing surface area) and variation, in chemical composition and in the chemical units' assembly (reducing cellulose crystallinity), so that the next step of hydrolysis can be enhanced by unblocking enzyme access to the cellulose (Abu-Thabit et al., 2022; Kumar et al., 2022; Vigneswari et al., 2021). The pretreatment also solubilizes hemicellulose and lignin into the liquid medium (Sidana and Yadav, 2022). Although the pretreatment is a key step for a proper LCB bioconversion, it entails some drawbacks that could make the biorefining process unsuccessful. For example, sugar degradation in organic acids or furfural due to harsh pretreatment conditions is common, or phenolic compounds derived from lignin disarray can even be generated. Most of these chemicals inhibit microbial growth and act as toxic agents for cells, which is an added difficulty of the subsequent bioconversion (Kumar et al., 2022). To counteract these toxic effects, the lignin content in LCB could be reduced, but detoxification is the usual alternative prior to hydrolysis, which increases the complexity and cost of the overall process.

The efficiency of the pretreatment depends largely on the type of biomass and lignocellulosic composition; some technologies solubilize lignin, others reduce the degree of cellulose crystallinity, or even hydrolyze hemicellulose completely. Conventional methods use pressure, heat, and chemical substances to break down the lignocellulosic polymer structures. These methods are classified as physical, chemical, physico-chemical and biological techniques (Fig. 2).

3.2.1. Physical pretreatments

Physical pretreatments results in a biomass with a higher surface area, smaller particle size, and lower crystallinity, making the next step in bioprocessing more efficient and easier (Brodeur et al., 2011). Physical treatment is considered environmentally friendly and does not generate toxic products; nevertheless, it is one of the methods that involves a high energy consumption, to the point of not being suitable in large-scale processes (Baruah et al., 2018). Milling, ultrasonication, extrusion or microwave treatment are examples of common physical methods. Milling or grinding reduces particle size down to 0.2 mm, but high energy requirements and the capital investment in mechanical equipment make its application for PHA production difficult. Extrusion consists of the combined action of both high temperature (>300 °C) and shear forces, which promotes the disruption of the recalcitrant structure of lignocellulose (Raj et al., 2022).

Microwave and ultrasound irradiation, considered as non-conventional heating methods, improve lignin solubility. It is often used in combination with chemical pretreatment methods, as Liu and colleagues showed that integrated microwave and alkaline treatment separated hemicellulose and cellulose from a delignified hardwood kraft pulp (Liu et al., 2018). They found that microwaves broke up the compact fiber structure and aided the penetration of the alkaline solution into the fiber structure, thus promoting hemicellulose removal and a cellulose content of 93.05%. The use of microwave pretreatment assisted with acid was tested for PHA production. Corn straw was treated with both microwaves and dilute hydrochloric acid, and later used as a carbon source for a mixed microbial culture for PHBV production. Two approaches were tested: a culture in shake flasks, where 41.4% of PHBV accumulation was reached, or in 3L-batch reactor, where biopolymer accumulation increased up to 76.3% (Verdini et al., 2022) (Table 1). Another approach using a different type of acid also converted dry spruce sawdust to the copolymer PHBV by *Cupriavidus necator* DSM 545, reporting 78.7% of PHBV with a volumetric productivity of 0.36 $gL^{-1}h^{-1}$ in shake flasks, and 75.3% of PHBV and 2.84 $gL^{-1}h^{-1}$ of volumetric productivity in a 7.5L reactor (Koller et al., 2015) (Table 1). Ultrasonication applies ultrasonic waves to biomass, which promotes the phenomenon of cavitation. The result is the generation of shear forces, high temperature, and oxidative radicals that cause the splitting of mantaining LCB linkages (Sidana and Yadav, 2022). Several factors determine the effectiveness of this method, such as sonication time, ultrasonic frequency, sonication power, temperature, as well as the type of solvent used (Baruah et al., 2018). For the production of LCB-derived PHA, ultrasonication is the most commonly used physical pretreatment, despite the limitations of the technique in terms of energy consumption, strict process monitoring, or phenolic acid by-products (Table 1).

3.2.2. Chemical pretreatments

Alkali pretreatment involves the use of hydroxides of sodium, potassium, calcium or ammonium and is based on lignin solubilization. Sodium hydroxide is the most commonly used solvent and has been

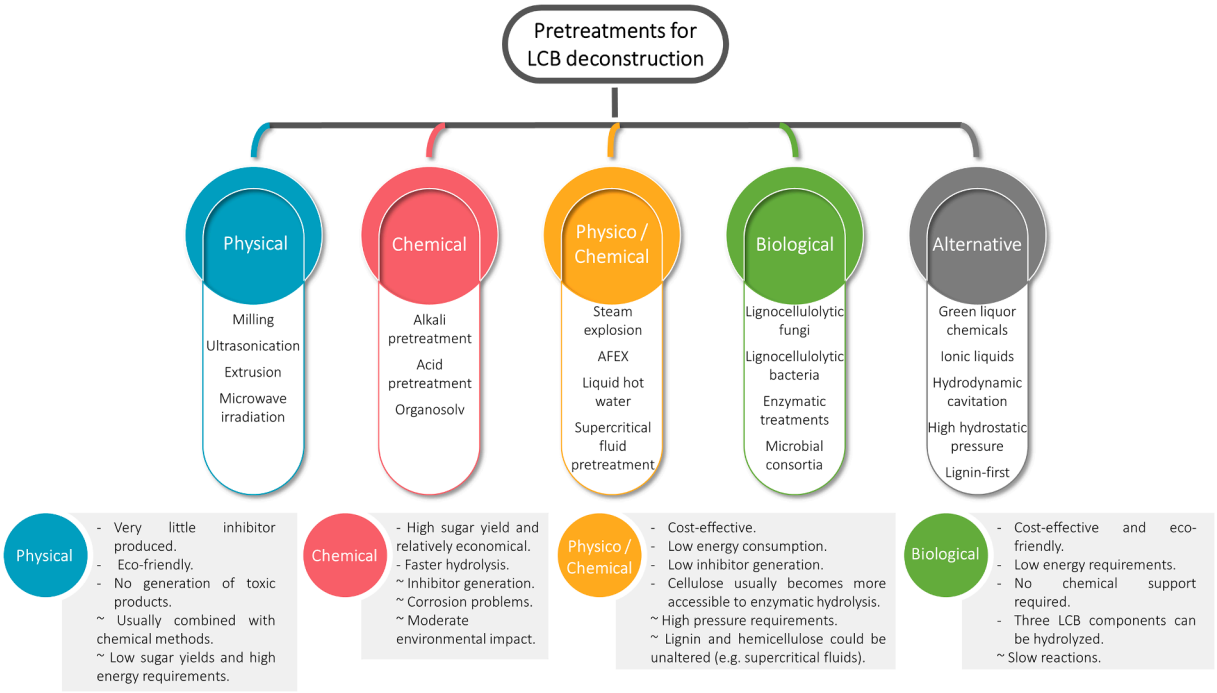

Fig. 2. Schematic diagram of the LCB pretreatment process.

employed for PHA production on a variety of biomass types, including wheat straw (Li and Wilkins, 2020), rice straw (Mohammad and Bhukya, 2022; Saratale and Oh, 2015), corn stover (Li et al., 2019), corn cobs (Khomlaem et al., 2023), and ryegrass (Davis et al., 2013) (Table 1). However, calcium hydroxide (lime) is a strong candidate owing to its low cost and safety. It has been shown to be effective in producing volatile fatty acids from LCB, which could be used as a carbon source for PHA production (Kim et al., 2013). In the form of a physical pretreatment, combined alkali treatment with other methods is very common and efficient (Table 1). Ultrasonic-assisted alkaline pretreatment has been applied to a variety of lignocellulosic materials for PHB production, such as wheat waste, millet straw, and agave leaves, achieving a polymer accumulation of up to 74.0% (Martínez-Herrera et al., 2021; Saratale et al., 2020; Silambarasan et al., 2021).

Glucosidic bonds between hemicellulose and cellulose are sensitive to acids and are the basis of an acid pretreatment to break down the rigid structures of LCB. Acids directly produce fermentable sugars, often eliminating the need for a subsequent enzymatic hydrolysis step. Acid pretreatment can be developed by using either concentrated or dilute solvents, such as sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), nitric acid (HNO_3), and also some organic acids (formic, malecic, or oxalic acids) (Baruah et al., 2018; Brodeur et al., 2011). This method consists of mixing biomass with concentrated or diluted acids at a moderate to high temperature. Typically, concentrated acids require moderate temperatures with associated corrosion and safety issues, while diluted acids work better at high temperatures. The main limitation of this pretreatment is the formation of undesirable compounds related to sugar degradation, such as furfural or 5-hydroxymethylfurfural, products of the degradation of pentose and hexose sugars, respectively, which significantly reduce the subsequent product yield (Al-Battashi et al., 2019). To control potential inhibitions, despite increasing operation costs, some approaches could be applied, including chemical, physical and biological detoxification methods, such as ion exchange resins, activated charcoal, flocculants, membrane separation, enzymatic detoxification, etc. (Al-Battashi et al., 2019; Baruah et al., 2018). The most commonly used solvent is dilute sulphuric acid, which has been employed to treat various types of biomass, such as sugarcane bagasse, maple, rice straw, or spent coffee grounds, amongs others, for PHA synthesis (Table 1). Interestingly, some studies have been reported in which PHA production could be achieved without applying any detoxification step. For example, this is the case of the approach developed by Saratale and coworkers, in which a co-culture of *Ralstonia eutropha* and *Lysinibacillus* sp. produced 72.1% PHA at a concentration of 10.25 g/L and at a volumetric productivity of 0.213 $gL^{-1}h^{-1}$ in shake flasks (Saratale et al., 2022).

3.2.3. *Physico-chemical pretreatments*

Among the physico-chemical pretreatments, steam explosion, ammonia fiber explosion (AFEX), liquid hot water and supercritical fluid pretreatment are the well-known techniques for LCB disintegration. Steam explosion subjects LCB to high pressure saturated steam and temperature for a short period of time, after which it suffers rapide depressurization, which promotes the breaking of the fibril structure. The combination of an acid catalyst with steam explosion is suitable for improving hemicellulose hydrolysis and the further enzymatic hydrolysis of cellulose (Brodeur et al., 2011). Similar to steam explosion, the AFEX pretreatment involves subjecting lignocellulosic material to high pressure and moderate temperature in liquid ammonia, followed by a suddend pressure release (Baruah et al., 2018; Paul et al., 2023). This methodology entails solvent recovery, lower energy consumption, and a reduction in overall associated costs. Liquid hot water solubilizes hemicellulose in the liquid fraction and discards lignin, making the cellulose more accessible (Baruah et al., 2018; Paul et al., 2023). Physico-chemical pretreatments have been less studied for PHA production, but various attempts to apply them to LCB valorization have been published in the literature with respect to wheat straw (Cesário et al., 2014), trembling aspen (Ramsay et al., 1995), pine tree wood chips (Bowers et al., 2014), waste office paper (Neelamegam et al., 2018), or even urban organic wastes (Allegue et al., 2022).

3.2.4. *Biological pretreatments*

Biological pretreatment uses lignocellulolytic bacteria and fungi (brown and white soft rot), microbial consortia and enzyme treatments as catalysts for lignocellulose degradation without the generation of inhibitory by-products. This approach is considered to be an efficient, cost-effective and eco-friendly alternative for the treatment of LCB

Table 1
Production of PHAs from different sources of LCB, subjected to a variety of pretreatments, by a diverse range of microorganisms.

Carbon source	Pretreatment	Microorganism	PHA type	PHA (gL^{-1})	PHA (%)	Yield (gg^{-1})	Productivity ($gL^{-1}h^{-1}$)	Scale	Ref.
Physical pretreatment									
Corn straw hydrolysate	Microwave-assisted acid hydrolysis	Mixed microbial culture from activated sludge (dairy industry)	PHBV	0.42 0.42	41.4 76.3	– –	0.0074 0.0052	Shake flasks Batch reactor	(Verdini et al., 2022)
Sawdust of spruce wood	Microwave-assisted acid hydrolysis	*Cupriavidus necator* DSM 545	PHBV	– 60.50	78.7 75.3	0.43 –	0.360 2.840	Shake flasks Batch reactor	(Koller et al., 2015)
Wheat waste biomass	Combined ultrasound and alkali	*Ralstonia eutropha* ATCC 17699	PHB	7.85	74.0	0.441	–	Shake flasks	(Saratale et al., 2020)
Finger millet straw	Ultrasound-assisted alkali (NaOH)	*Bacillus megaterium* CAM12	PHB	7.85	–	–	–	Shake flasks	(Silambarasan et al., 2021)
Agave durangensis leaves	Ultrasound followed by thermal treatment	*Bacillus cereus* 4 N	PHB	0.66	39.99	–	–	Shake flasks	(Martínez-Herrera et al., 2021)
Chemical pretreatment									
Black liquor from sugarcane bagasse	Alkaline pretreatment	*Pseudomonas monteilii* BCC 19149	PHBD	0.24	13.00	–	–	Fed-batch reactor	(Unrean et al., 2023)
Lignin from rice straw	Alkali (NaOH)	*Pseudomonas putida* DT2440	PHA	1.26	57.00	–	–	Shake flasks	(Mohammad and Bhukya, 2022)
Rice paddy straw hydrolysate supplemented with corn steep liquor	Alkali (NaOH)	*Ralstonia eutropha* ATCC 17699	PHB	11.42	75.45	–	–	Shake flasks	(Saratale and Oh, 2015)
Corn stover hydrolysate	Alkali (NaOH)	*Cupriavidus necator* DSM 545	PHB	2.12	–	–	–	Shake flasks	(Li et al., 2019)
Ryegrass hydrolysate	Alkali (NaOH)	*Pseudomonas putida* W619 *Pseudomonas putida* KT2440 *Pseudomonas fluorescens*	*mcl*-PHA	–	25.70 23.40 34.50	–	–	Shake flasks	(Davis et al., 2013)
Corn cob hydrolysate	Alkali (NaOH)	*Bacillus megaterium* ALA2	PHB	5.80 57.60	50.30 56.60	–	0.276 1.07	Shake flasks Reactor (cell retention)	(Khomlaem et al., 2023)
		Paracoccus sp. LLI	PHBV	3.46 31.30	39.70 44.50		0.144 0.579	Shake flasks Reactor (cell retention)	
Rice straw hydrolysate	Alkali (NaOH)	*Pseudomonas putida* KT2440	PHB	0.47	47.00	–	–	Shake flasks	(Hossain et al., 2022)
Hemp hurd hydrolysate	Alkali (NaOH)	*Pseudomonas putida* KT2440	PHB	0.61	69.00	–	–	Shake flasks	(Hossain et al., 2022)
Rice husk hydrolysate	Alkali (KOH)	*Burkholderia cepacia* USM (JCM 15050)	PHA	2.35 –	62.00 50.00	–	–	Shake flasks Batch reactor	(Heng et al., 2017)
Rice straw hydrolysate	Alkali (ammonia)	*Bacillus cereus* VK92 *Bacillus cereus* VK98	PHA	2.96 2.51	59.30 46.40	0.15 0.13	0.062 0.052	Shake flasks	(Van Thuoc et al., 2021)
Rich cotton stalk hydrolysate	Acid (HNO_3)	*Pseudomonas putida* KT2440	PHD/PHO	0.24	–	–	–	Shake flasks	(Bellary et al., 2023)
Palm empty fruit bunch hydrolysate	Acid (HNO_3)	*Pseudomonas putida* KT2440	PHD/PHO	0.21	–	–	–	Shake flasks	(Bellary et al., 2023)
Sugarcane bagasse hydrolysate	Acid (H_2SO_4)	*Lysinibacillus* sp. and *Ralstonia eutropha*	PHA	10.25	72.10	0.406	0.213	Shake flasks	(Saratale et al., 2022)
Rice straw hydrolysate	Acid (H_2SO_4)	*Bacillus megaterium* B-10	PHB	1.50	32.56	–	–	Shake flasks	(Li et al., 2021)
Spent coffee grounds hydrolysate	Acid (H_2SO_4)	*Halomonas halophila* CCM 3662	PHB	0.95	27.00	–	–	Shake flasks	(Kovalcik et al., 2018)
Spent coffee ground hydrolysate	Acid (H_2SO_4)	*Burkholderia cepacia*	PHBV	2.69	54.79	0.230	–	Shake flasks	(Obruca et al., 2014a)
Rice straw waste hydrolysate	Acid (H_2SO_4)	*Cupriavidus necator* H16	PHBV	0.92	–	–	–	–	(Ahn et al., 2016)
Sugar maple hemicellulose hydrolysate	Acid (H_2SO_4)	*Burkholderia cepacia* ATCC 17759	PHB	8.72	51.7	0.19	–	Fed-batch reactor	(Pan et al., 2012)
Apple residues hydrolysate	Acid (H_2SO_4)	*Azotobacter vinelandii* ATCC9046	PHB	– 3.94	– 67.30	0.160 0.160	0.012 0.054	Shake flasks Batch reactor	(Andler et al., 2023)
Mango peel hydrolysate	Without pretreatment Acid (H_2SO_4)	*Bacillus thuringiensis* IAM 12077	PHB	4.03 3.30	51.30 45.60	0.100 –	0.168 –	Shake flasks	(Gowda and Shivakumar, 2014)
Jackfruit seed powder hydrolysate	Without pretreatment Acid (H_2SO_4)	*Bacillus thuringiensis* IAM12077		3.93 8.03	29.32 51.70	– 0.100	– 0.163		

(*continued on next page*)

Table 1 (*continued*)

Carbon source	Pretreatment	Microorganism	PHA type	PHA (gL^{-1})	PHA (%)	Yield (gg^{-1})	Productivity ($gL^{-1}h^{-1}$)	Scale	Ref.
Oil from spent coffee grounds	n-hexane pretreatment	*Cupriavidus necator* H16	PHB	10.30 49.40 26.50	75.60 89.10 90.10	– 0.880 0.820	– 0.660 1.330	Shake flasks Batch reactor Fed-batch reactor	(Obruca et al., 2014b)
Corn stover hydrolysate	Alkaline H_2O_2	*Paracoccus* sp. LL1	PHB	9.71	72.40	0.250	0.134	Batch reactor	(Sawant et al., 2015)
Lignin from rice Straw biomass	H_2O_2-homogenizer pretreatment	*Bacillus cereus*	PHA	0.480	56.00	0.561	–	–	(Kavitha et al., 2023)
Waste office paper hydrolysate	H_2O_2 pretreatment + autoclave	*Cupriavidus necator* NCIMB 11599	PHB	4.45	57.52	0.210	0.060	Shake flasks	(Neelamegam et al., 2018)
Waste office paper hydrolysate	H_2O_2 pretreatment + autoclave	*Burkholderia sacchari* DSM 17165	PHB	–	44.20	–	–	Shake flasks	(Al-Battashi et al., 2019)
VFAs from waste office paper hydrolysate	H_2O_2 pretreatment + autoclave + anaerobic digestion	*Cupriavidus necator* DSM 428	PHB	–	53.50	0.200	–	Shake flasks	(Al Battashi et al., 2021)
Physico-Chemical pretreatment									
Ensiled perennial ryegrass	Screw press pretreatment + autoclave	*Burkholderia sacchari* IPT 101	PHB	– 13.80	23.30 35.00	– 0.470	– 0.380	Shake flasks Fed-batch reactor	(Cerrone et al., 2015)
		Pseudomonas chlororaphis IMD 555	*mcl*-PHA	3.75	10.00	0.100	–	Fed-batch reactor	
Hemicellulose from trembling aspen	Steam explosion	*Pseudomonas cepacia* ATCC 17759	PHB	–	60.00 45.00	– 0.110	–	Shake flasks Batch reactor	(Ramsay et al., 1995)
Corn stover	Steam explosion	*Baciullus megaterium* PNCM 1890	PHB	1.81	–	0.190	–	Shake flasks	(Perez et al., 2023)
Urban organic waste hydrolysate	Steam explosion and acidogenic fermentation	Mixed culture of purple phototrophic bacteria	PHB	–	42.00	–	–	Batch reactor	(Allegue et al., 2022)
Pinnus radiata wood chips	Steam explosion with Sulphur dioxide	*Sphingobium scionense* WP01	PHB	0.25	17.00	0.050	–	Shake flasks	(Bowers et al., 2014)
Wheat straw hydrolysate	AFEX	*Burkholderia sacchari* DSM 17165	PHB	4.40 –	57.00 72.00	0.190 0.220	– 1.600	Shake flasks Fed-batch reactor	(Cesário et al., 2014)
Biological pretreatment									
Corn husk hydrolysate	*Pleurotus ostreatus* MTCC 142	*Bacillus megaterium Ti*3	PHB	1.00	57.80	0.100	0.021	Shake flasks	(de Souza et al., 2020)
Sugarcane bagasse hydrolysate	Biological pretreatment	*Burkholderia cepacia* IPT101	PHB	–	62.00	0.390	0.011	Shake flasks	(Silva et al., 2004)
Other alternatives for pretreatment									
Hibiscus cannabinus L. (kenaf) hydrolysate	Emerging chemical pretreatment (Na_2CO_3 and Na_2SO_3)	*Ralstonia eutropha*	PHB	13.93	71.90	0.464	–	Shake flasks	(Saratale et al., 2019)
Paddy straw hydrolysate	Alkali + Acid pretreat. Followed by treatment with enzyme form *Aspergillus nidulans*	*Burkholderia gladioli* 2S4R1 *Bacillus cereus* LB7	PHB	16.98 12.76	26.80 20.47	–	–	Shake flasks	(Naitam et al., 2022)
Kraft lignin	Kraft process	*Cupriavidus basiliensis* B-8	PHB	0.13 0.32	–	–	–	Shake flasks Fed-batch reactor	(Shi et al., 2017)
Vegetable waste	Hydrodynamic cavitation	*Cupriavidus necator* DSM 545	PHB	–	44.00	0.370	–	Fed-batch reactor	(Lanfranchi et al., 2022)
Brewer's spent grains	Non-thermal plasma	*Paraburkholderia sacchari* DSMZ 17165	PHB	95.60	58.80	0.270	2.770	Fed-batch reactor	(Argeiti et al., 2022)

(Baruah et al., 2018). Whether the whole microorganism is used or only the enzyme cocktail is selected, the enzymes responsible for lignin degradation are laccase, lignin peroxidase, manganese peroxidase and versatile peroxidase. Regarding specific lignocellulolytic microorganisms, Basidiomycota is the Phylum that habors the best lignin degraders, such as white rot fungi, because they have these specific enzymes (Kumar et al., 2022). On the contrary, bacteria are not as efficient lignin degraders, although their fast growth and metabolism make them a potential alternative to fungi. Among bacteria, there are specific genera isolated mainly from the gut microbiota, such as *Fibrobacter*, *Ruminococcus*, *Clostridium*, which are able to utilize cellulose and hemicellulose and degrade them into soluble carbohydrates. These microorganisms have a special system for cellulose degradation called a carbohydrate-active enzyme (CAZyme), in which cellulase is classified (Froidurot and Julliand, 2022; Kumar et al., 2022). There are limited studies on PHA production from hydrolysates of biologically pretreated LCB; however, some attempts have been made using different feedstocks, such as corn husk, rice straw, sugarcane bagasse, newspaper, and wheat bran, among others (de Souza et al., 2020) (Table 1). Specifically, the biological action of *Pleurotus ostreatus* on corn husk allowed a PHB accumulation of up to 57.8% by *Bacillus megaterium* (de Souza et al., 2020). Moreover, some studies have tried to discover specific microorganisms with both abilities: lignin degradation and simultaneous PHA synthesis. In this regard, *Oceanimonas doudoroffii*, a marine bacterium, is

capable of directly synthesizing PHAs using lignin or its derivatives as the sole carbon source (Numata and Morisaki, 2015).

3.2.5. Alternative pretreatments

Today alternatives to LCB deconstruction have emerged, mainly because traditional processes do not guarantee a sustainable development of LCB-derived products. In this regard, some emerging chemical pretreatment methods include the use of green liquor chemicals, such as sodium carbonate (Na_2CO_3) and sodium sulfite (Na_2SO_3), which can break lignin and hemicellulose ester bonds, and induce lignin sulfonation, respectively. Saratale and colleagues showed a significant improvement by using of a mixture of Na_2CO_3 and Na_2SO_3 for the pretreatment of kenaf biomass. A maximum PHB accumulation of 71.9% in *Ralstonia eutropha*, with a PHB titer of 13.93 g/L and PHB yield of 0.464 g/g were reported (Saratale et al., 2019). Moreover, ionic liquids (ILs) represent a new class of cellulose solvents. ILs are salts with melting point below 100°C, composed of cations and anions, with very attractive properties such as being recoverable, reusable, safe and non-toxic compound producers (Brodeur et al., 2011). Hydrodynamic cavitation is another developing technique that promotes delignification (Sidana and Yadav, 2022). It has been applied to PHA synthesis by *Cupriavidus necator* DSM 545, achieving an accumulation of up to 44% of PHB from vegetable waste (Lanfranchi et al., 2022). In addition, the lignin-first approach is an alternative strategy that extracts lignin from biomass by solubilizing it into small molecules. Lignin-first employs solvolytic extraction, followed by lignin depolymerization by the catalytic conversion of reactive intermediates or the protection of reactive aromatics, which improves the conversion rate of lignin (Abu-Omar et al., 2021). Efforts have been made to make the process more sustainable, as it is extensively reviewed by Korányi and colleagues, who showed the evolution of this approach and future perspectives that make lignin-first a promising strategy for a future PHA synthesis (Korányi et al., 2020). High hydrostatic pressure and high-pressure homogenization are alternative techniques that have not been described as being applied for PHA production; however, they have been used for LCB deconstruction, as reviewed elsewhere (Sidana and Yadav, 2022). Currently, limited data are available for economic and energetic cost assessment of these alternative technologies, but they represent a promising line of research to be explored for a sustainable future of LCB valorization.

3.2.6. Enzymatic hydrolysis

After LCB pretreatment, enzymatic hydrolysis is commonly applied to hydrolyze cellulose and hemicellulose into sugars for PHA production. Complete cellulose hydrolysis is achieved by the synergistic action of three types of cellulases, which cleave the β-(1–4)-D-glucose. In particular, cellulases are classified according to their mode of action as endoglucanases (or carboxymethylcellulases, which cleave at a random position in the chain), exocellobiohydrolases (which release cellobiose), and β-glucosidase (which hydrolyzes cellobiose or small oligomers) (Froidurot and Julliand, 2022). Hemicellulose hydrolysis requires more enzymes for complete monosaccharide release, such as endoxylanase, exoxylanase, β-D-xylosidase, acetylxylan esterase, α-glucuronidase, α-arabinofuranosidase and ferulic acid esterase (Abu-Thabit et al., 2022). Inhibitory products from the pretreatment, as well as the lignocellulosic hydrolysate itself, could inhibit the enzymatic activity and reduce its efficiency. Some lignin-derived compounds, such as vanillin, syringaldehyde, furfural or hydroxymethylfurfural, deactivate the cellulase. Moreover, the negative feedback exerted by glucose or cellobiose significantly delays the cellulose hydrolysis (Al-Battashi et al., 2019). These drawbacks, together with its slowness and high cost, make it difficult to apply enzymatic hydrolysis on an industrial scale, despite its eco-friendly nature.

3.3. PHA production from lignocellulosic feedstocks

Recent advances in PHA synthesis from LCB have focused on improving culture conditions to achieve refined yields, evaluating the best parameters for improving PHA-producing bacterial culture. In this way, the most common operating modes found in the literature are batch (both in shake flasks and bioreactors), fed-batch, and continuous culture, with slight modifications. Despite the low cost and versatility of batch culture, the usual mode of operation on an industrial scale is fed-batch culture. The reason is simple; this system allows high cell density and also a high yield and productivity.

So far, although there is a wide range of PHA-based bioplastics, PHB and PHV, along with their co-polymer PHBV, are the most typical biopolymers produced from LCB (Table 1). In addition to the mode of operation, one of the main factors affecting PHA production is the PHA-producing microorganism used (pure cultures or mixed microbial culture). To date, hundreds of microorganisms have been studied with the ability to produce PHAs in different ranges. However, if a reference microorganism must be cited, the candidate would be *Cupriavidus necator*, which has been used extensively both at laboratory scale and industrial scale, and it was the bacteria that allowed the study of the PHA biosynthetic pathway (Kutralam-Muniasamy and Peréz-Guevara, 2018). Furthermore, the type of carbon source (simple sugars or more complex materials) defines not only the PHA production yield, but also the polymer composition.

In this regard, PHA synthesis from lignocellulosic materials has been explored in a wide variety of renewable feedstocks (Table 1). *Bacillus megaterium* PNCM 1890 was able to produce 1.81 g/L of PHB and 0.1872 g/g (in terms of reducing sugars consumed) from corn stover hydrolysate in a shake flask system (Perez et al., 2023). However, the PHB yield can be improved in a batch reactor, as shown by Sawant and colleagues. They cultured *Paracoccus* sp. LL1 in corn stover hydrolysate in a 5L-batch reactor, yielding 9.71 g/L of PHB and 0.251 g/g (Sawant et al., 2015). Other studies have attempted to improve PHA yield by adding levulinic acid to corn stover hydrolysate. However, depending on the bacterial strain, the effect could be detrimental, as was the case with *Burkholderia sacchari*, which reduced PHBV synthesis by half when this additive was present, reaching only 1.2 g/L of PHBV. In contrast, *Azohydromonas lata* doubled the production of a PHA terpolymer, when levulinic acid was added to corn stover hydrolysate, producing 1.0 g/L of PHBV with 4-hydroxyvaleric acid (Ashby et al., 2022). In addition, corn derivatives, such as corn steep liquor, could improve PHA yields as a nitrogen source, increasing the production rate of PHBV from 3.7 g/L to 19.5 g/L after sugarcane molasses supplementation in shake flasks (Ojha and Das, 2018). Moreover, supplementation of rice paddy straw hydrolysate with corn steep liquor maximized PHB accumulation by *Ralstonia eutropha* ATCC 17699 (75.45% and 11.42 g/L), indicating that this supplement could be a good nutritional additive (Saratale & Oh, 2015).

Wheat by-products have been explored as feedstock for PHA production. *Bacillus thuringiensis* SBC4 accumulated 18.75% PHA using wheat bran hydrolysate as carbon source in shake flasks; although the highest PHA accumulation was achieved using corn cobs (21.05% PHA) (Odeniyi and Adeola, 2017). In addition, wheat straw hydrolysate was used for PHB synthesis using *Burkholderia sacchari* DSM 17165, rendering 4.4 g/L of PHB and 57% accumulation in shake flask experiments (Cesário et al., 2014) (Table 1). Surprisingly, the same authors obtained up to 105 g/L of PHB by changing the mode of operation to fed-batch in a 2L stirred tank reactor, yielding 72% of PHB and a volumetric productivity of 1,6 $gL^{-1}h^{-1}$ (Cesário et al., 2014).

Recently, the number of studies on the valorization of rice-based lignocellulosic materials into PHAs has increased rapidly (Table 1). Most have been carried out at laboratory scale, without any development by scaling-up process to a higher scale. PHA production varies from one study to another, ranging from 0.47 g/L of PHB produced by *Pseudomonas putida* KT 2440 to 2.96 g/L of PHA produced by *Bacillus cereus* using rice straw hydrolysate (Ahn et al., 2016; Hossain et al., 2022; Kavitha et al., 2023; Li et al., 2021; Mohammad and Bhukya, 2022; Van Thuoc et al., 2021). The low productivity yield could be due

to the chosen mode of operation, as all of these attempts were conducted in shake flasks. Furthermore, the valorization of rice husks into PHAs by *Priestia megaterium* POD1 yielded 5.13 g/L of PHA in shake flask cultivation (Sehgal et al., 2023), which is higher than previous reports, where only 2.35 g/L of PHA was obtained using the same regime (Heng et al., 2017).

Other carbon sources based on lignocellulosic materials, such as hydrolysates of spent coffee grounds, sawdust, wood, fruit peelings, or even waste paper, have been subjected to various microbial cultures for PHA production (Table 1). *Burkholderia sacchari* is a potential PHA producer that is able to metabolize waste paper hydrolysate into PHB, accumulating a maximum of 3.95 g/L when grown in shake flasks (Al-Battashi et al., 2019). The same authors included anaerobic digestion as part of the pretreatment applied to waste office paper, obtaining volatile fatty acids that were used for PHB synthesis by *Cupriavidus necator* DSM 428. In this approach, developed in shaking flasks, 53.50% of the PHB was accumulated inside the cells (Al-Battashi et al., 2019). Interesting results on PHA production from fruit waste were shown by Gowda and Shivakumar, 2014, who demonstrated the possibility of avoiding the acid pretreatment of LCB by using *Bacillus thuringiensis* IAM 12077 as PHA-producing bacteria. They managed to increase the PHB accumulation compared to the corresponding acid-pretreated feedstock when using untreated mango peel. Nevertheless, they increased the PHB yield when acid-pretreated jackfruit seed powder was used as feedstock (Table 1). *Klebsiella pneumoniae* isolated from the paper-manufacturing industry was described as having the ability of PHB accumulation when different fruit peels (banana, orange, papaya, watermelon and melon) were used as feedstock, ranging from 26.40 to 32.90 % of PHB (Valdez-Calderón et al., 2022). The addition of volatile fatty acids was shown to improve PHA production. Fruit pulp waste was found to give acceptable results for PHBV synthesis in a pilot scale bioreactor. In this sense, a mixed microbial culture was selected from an activated sludge from a municipal wastewater treatment plant and fed to a 100L fed-batch reactor with volatile fatty acids derived from acidogenic fermentation of fruit pulp waste. The authors reported a maximum PHBV accumulation after the last feeding pulse of 6.16 g/L or 88.95% (Sousa et al., 2021).

In addition, the membrane bioreactor is another mode of operation recently described for PHA production from LCB. Corn cob hydrolysate was used to co-produce not only PHA but also astaxanthin, a high-demand carotenoid with a high market value, making the process more attractive for biorefinery integration (Khomlaem et al., 2023) (Table 1). Moreover, this study showed the highest rates of PHA accumulation, reporting 57.6 g/L and 31.3 g/L, using the cell retention system of *Bacillus megaterium* ALA2 and *Paracoccus* sp. LL1, respectively. These data were approximately nine times higher than those reported by batch culture (Khomlaem et al., 2023).

As can be observed, although some attempts for PHA production from lignocellulosic materials have been described with very attractive yields, many are still below the values required for scaling up to pilot scale. Obviously, yields are determined by several factors, not only the type of LCB. The mode of operation and the microorganism strain also have a major impact on process yields. Another critical factor for low productivity is the so-called carbon catabolite repression, which consists of a global regulatory mechanism that controls the use of carbon sources when some of them are present in the culture medium; so, the microorganism selectively uses one of the substrates, making the PHA synthesis process more complex and therefore with a low productivity. It should not be forgotten that LCB pretreatment, which is often indispensable, generates organic acids and lignin derivatives that could inhibit the growth of PHA-producing bacteria. All this seems to indicate that, although PHA production from LCB still requires further research efforts, improvements and optimization, some of the results obtained to date postulate LCB as a promising feedstock in the very near future.

3.4. Biological strategies for improving PHA production from LCB

The bioconversion of lignocellulosic waste by microbial metabolism for the production of PHAs is a strategy that supports the circular economy. However, throughout this review, some problems related to this transformation have been underlined that, to some extent, make the feasibility of an industrial process from this particular feedstock uncertain. In order to overcome these difficulties, different biological strategies have been developed that allow some form of PHA synthesis.

Co-culture is a biological system in which two or more different strains of microorganisms are grown together. The population established in a co-culture system can cooperate or compete under certain conditions (Khatami et al., 2022), with cooperation being the desired relationship when the goal is LCB bioconversion. Co-culture could counteract some of the drawbacks associated with the pretreatment of lignocellulosic materials, as this approach could neutralize toxic metabolites, promote enzymatic hydrolysis, or support the synthesis of a specific type of PHA. Specifically, co-culture has recently been used for the bioconversion of *Miscanthus* grass, corn stalk and corn leaves into PHAs (Kumar et al., 2023). Two different strains were used in this study: *Streptomyces* sp. SirexAA-E and *Priestia megaterium* NBRC 15308 (formerly known as *Bacillus megaterium*), with high cellulolytic activity and the ability to produce PHAs, respectively. None of the strains alone were able to produce PHAs from LCB; however, co-culture allowed PHB production from the three feedstocks as the sole carbon source, with *Miscanthus* grass providing the highest production rate (0.20 g PHB/L and 0.040 g/g). It should be noted that both corn stalk and corn leaves allowed PHB production, but to a lesser extent; this could be due to the fact that the grass biomass was previously alkali pretreated, whereas the corn by-products were not. Furthermore, co-culture could employ bacterial strains with PHA synthesis ability in order to carry out an effective utilization of LCB, promoting a synergistic effect of the different microorganisms. This is the case reported by Saratale and colleagues, who grew *Lysinibacillus* sp. and *Ralstonia eutropha*, both known PHA producers, on a medium containing sugarcane bagasse hydrolysate as a carbon source (Saratale et al., 2022). They showed a PHA rate of 10.25 g/L and a PHA accumulation of 72.1% when using 30 g/L of reducing sugars from the hydrolysate (Table 1). They also tried supplementing the media with other lignocellulosic hydrolysates, such as corn steep liquor and spent coffee waste oil, which increased the PHA concentration up to 8.87 g/L and 8.36 g/L, respectively (relative to the control at 20 g/L of reducing sugars) (Saratale et al., 2022). On other occasions, co-culture may serve to overcome the inhibition of sugar utilization. This is the case reported by Lee and coworkers, who isolated a xylose-utilizing PHB producer (*Bacillus* sp. SM01). To avoid the inhibition of this strain by the presence of glucose, they performed a co-culture with *Cupriavidus necator* NCIMB 11599 (a well-known PHB producer that is unable to utilize xylose) (Lee et al., 2021). They defined a chemical medium whose composition mimicks lignocellulosic biosugars and showed a raise in PHB production of 40% in the co-culture, as compared to the corresponding value for the monoculture, reaching 3.55 g/L of PHB titer.

Another biological strategy to improve the valorization of lignocellulosic wastes is genetic engineering. This field of molecular biology would allow the introduction of new and diverse functionalities in a microorganism with different purposes, such as the elimination of pretreatment or detoxification process of LCB or the enhancement of PHA production, among many others. In this regard, next-generation sequencing approaches have served to address various issues related to PHA and lignin synthesis and degradation mechanisms, not only at the molecular level, but also on a larger scale, including the complete biosynthetic/catabolic pathways. In this sense, metagenomics has been used to characterize new unculturable PHA-producing microorganisms and to map carbohydrate-degrading enzymes, such as the CAZyme family or enzymes associated with lignin metabolism (Kumar et al., 2022; Paul et al., 2023). Through genetic engineering, it is possible to obtain a microorganism with a combined lignocellulose-degradating

function and PHA-producing ability, or to make a host strain capable of growing in the presence of certain inhibitors contained in the LCB hydrolysate. To enhance PHA synthesis in *Halomonas elongata* A1, a PHB-producing bacterium, a genetic modification was developed using a plasmid-based system to introduce the three key genes *phaC*, *phaA* and *phaB*. The resulting engineered bacteria increased the PHB yield 4-fold over the wild type. In addition, the improved strain was able to metabolize wheat straw hydrolysate up to 5.2% of PHB (0.44 g/L), a value that increased up to 16.5% of PHB (1.28 g/L) when oleic acid was used as a supplement (Liu et al., 2021). Moreover, the composition of the biopolymer could be modulated by an engineered strain, as in the case of a recombinant *Escherichia coli* that fully utilized xylose and glucose. This strain was engineered to harbor key genes for PHA synthesis and was able to synthesize a poly-(3-hydroxybutyrate-co-lactate) [P(3HB-*co*-LA)] using corn stover hydrolysate as a carbon source, accumulating approximately 60% of the polymer (Wu et al., 2021). In a previous approach, *Escherichia coli* was metabolically modified to assimilate xylose and produce PHAs. The resulting transformant produced P(3HB-*co*-LA) at a final concentration of 3.7 g/L (40.4% of intracellular accumulation) when beechwood xylan was used as a carbon source in combination with xylose (Salamanca-Cardona et al., 2014). Moreover, metabolic engineering was successfully applied to *Ralstonia eutropha* NCIMB 11599 to express the *Escherichia coli xylAB* genes, responsible for pentose utilization. Using sunflower stalk hydrolysate, the enhanced bacteria accumulated 72.53% of biopolymer and produced 7.86 g/L of PHB (Kim et al., 2016). A combination of strategies could be used, as was done by co-culturing with two engineered microorganisms. For example, *Pseudomonas putida* KTΔABZF (p2-a-J), capable of synthesizing PHA, and *Escherichia coli* Δ4D (ACP-SCLAC), with the ability of producing free fatty acids, were grown simultaneously in such a way that the latter provided nutrients for the former. This microbial consortium produced 1.02 g/L of *mcl*-PHAs using corn straw hydrolysate (Qin et al., 2022).

New genome editing tools, such as clustered regularly interspaced short palindromic repeats (CRISPR)-based tools, have revolutionized the field of genetic engineering. In the near future, it is possible that this methodology could be used to generate new strains with an enhancement for LCB-to-PHA bioconversion. This novel technique, long known as a defense immune system in bacteria, has been successfully used to adapt metabolisms toward PHA biosynthesis. Using this method, Zhou and colleagues developed a genetically engineered *Pseudomonas putida* KT2440 variant that overcame the growth inhibition exerted by ferulic acid and produce of 270 mg/L of mcl-PHA using this toxic compound (Zhou et al., 2020).

4. Concluding remarks

PHAs have emerged as a viable alternative to petroleum-based plastics. However, in order to achieve a feasible industrial development, the production costs should be significantly reduced. To accomplish this goal, renewable feedstocks have been proposed as cheap carbon sources to be used as raw materials for PHA synthesis. In particular, lignocellulosic biomass is a promising feedstock for PHA production due to its abundance and underutilization. Its industrial use is still under development because of several obstacles that make the process economically unviable. To date, significant progress has been made in the field of pretreatment and enzymatic hydrolysis, the previous steps in microbial conversion to PHAs. In addition, the optimization of microbial culture conditions and the selection of specific PHA-producing microorganisms have yielded promising results, although they are still at the research stage. Moreover, biological strategies are pioneering the field with the aim of avoiding these initial steps or even of obtaining an improved microorganism capable of the synergistic biotransformation of lignocellulosic material into PHAs. In conclusion, the valorization of lignocellulosic by-products into high value-added compounds could make the cost-effective industrial production of PHAs a reality. Scaling up biotransformation from this type of feedstock requires further research efforts to become a truly economically attractive and sustainable process that contributes to the circular economy.

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.

Data availability

No data was used for the research described in the article.

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