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Perspective

Valorization of Lignocellulose by Producing Polyhydroxyalkanoates under Circular Bioeconomy Premises: Facts and Challenges

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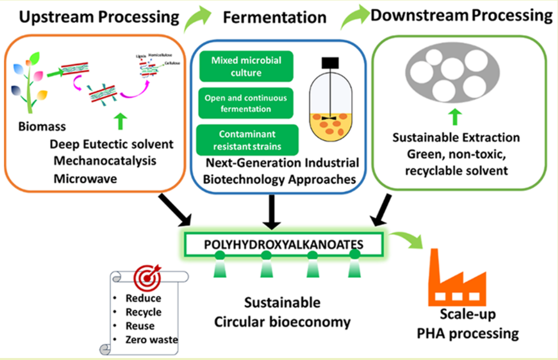
ABSTRACT: In light of the current concerns about environmental issues caused by the excessive use of fossil resources, more emphasis has been paid to the transition to a sustainable and circular economy. Bioplastics as eco-friendly products originating from biomass wastes have gained much attention to solve the problem of plastic pollution. Among them, polyhydroxyalkanoates (PHAs) are microbial polyesters produced using various feedstocks—renewable or recycled waste materials—contributing to a more sustainable commercial plastic life cycle by being a part of a circular bioeconomy. However, the scale-up of the PHA process cost effectively and sustainably remains challenging for large-scale industrial applications. This perspective provides a comprehensive overview of the current insights into lignocellulosic biomass's role in achieving a circular bioeconomy. Emerging greener biomass conversion technologies are discussed to characterize energy demand, cost, and sustainability within biorefinery PHA production. In addition, recent advances in synthetic biology and fermentation processes for PHA production are discussed. Technological challenges, i.e., bioreactor setup, downstream operation, and inconsistent properties to improve the sustainable production of PHAs and to help transfer this technology to real-world applications, are also addressed.



KEYWORDS: *Circular bioeconomy, Closed-loop biorefinery, Polyhydroxyalkanoates, Greener pretreatments, Fermentation strategies, Downstream strategies, Sustainability assessment*

■ INTRODUCTION

Fossil-based plastics are inexpensive, durable, lightweight, readily processable, and widely used in various industrial applications such as packaging, electronic appliances, automotive parts, etc.[1−3] However, in recent years, the inevitable increase in plastic consumption and disposal has caused solid waste pollution in nature.[4] It is estimated that more than 150 million tons of plastics waste is floating on the surface of the sea. Approximately 8 million tons of plastics are dumped in the ocean yearly, equivalent to a 1-ton garbage truck per minute.[5] This plastic waste concern becomes worse by the excessive use and disposal of fossil-derived plastic products, in a linear disposal pattern, showing the need of changing a usual linear business pattern to a more sustainable solution.[6] The circular economy is based on the zero waste concept in which waste generated after producing one product can be used as feedstock for another loop of production.[6] A closed-loop stream of waste material through a circular economy approach reduces the demand for the new raw material by maximizing the values of the resources.[7] Despite some fossil-based plastics being recyclable, such as polyethylene terephthalate (PET), this shows many obstacles such as formation of microplastics. Recently, microplastics have raised serious concerns since they are deposited in the tissues of aquatic organisms and thereby enter the food chain, leading to damage of aquatic life and ultimately human life.[6,8] Therefore, there is a need for urgent development of greener alternatives to fossil-based plastics.[9−11] Since plastics are a vital part of modern society,[12] extensive research is ongoing, focusing on the production of green and sustainable plastics.[3] These plastics are mainly made from renewable feedstocks that naturally degrade in the environment without leaving any toxic substance (bioplastics).[13−16] Emerging bioplastics with sustainable features are PHA bioplastics, which have attracted an increasing scientific community's attention due to their biocompatible and biodegradable natures.[17−19] PHAs are polyesters synthesized by three routes, namely, chemical, enzymatic, and microbiological.[20] In the chemical route, the most common strategy is ring-

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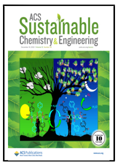

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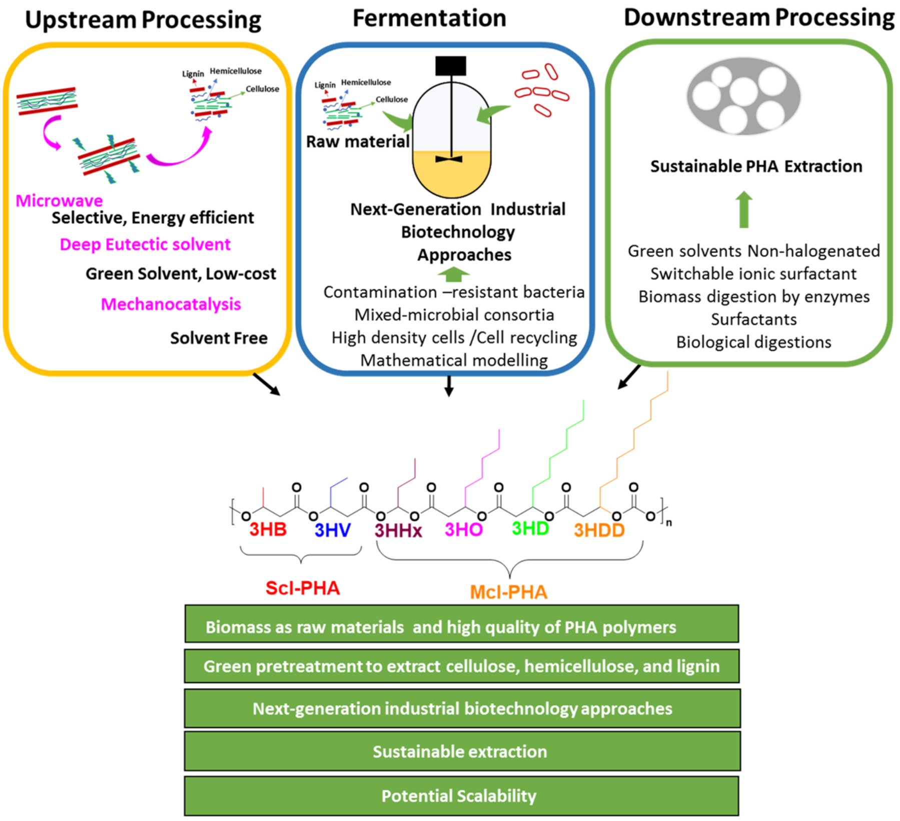

Figure 1. Emerging technologies for sustainable processing of lignocellulosic biomass toward PHA production: biomass pretreatments, fermentation, and downstream. The green boxes show the four central topics for critical discussion in this perspective. 3HB, 3-hydroxybutyrate; 3HV, hydroxyvalerate; 3HHX, 3-hydroxyhexanoate; 3HO, hydroxyoctanoate; 3HD, hydroxydecanoate; 3HDD, hydroxydodecanoate.

opening polymerization (ROP). In this process, metal catalysts such as magnesium dibutyl catalyst, tert-Bu-P4, diphenyl phosphate, and initiators such as alcohols are used for the polymerization of lactones into linear PHA polyester.[21] Enzyme-catalyzed in vitro synthesis of PHAs is promising route as it enables higher control over monomer compositions in the copolymer and molecular weight, and unlike bacteria, it does not accumulate PHA inside their cells. Hence, this approach simplifies the PHA downstream process.[22] However, the enzymatic route to synthesis of PHA is more expensive because it requires a costly precursor (R)-3-hydroxybutyryl-CoA and purified PHA synthase.[23] In contrast, microbial PHA production is less expensive compared to enzymatic and chemical routes. Therefore, the microbiological route for PHA synthesis has received more attention. In this route, PHAs are synthesized by prokaryotic microorganisms as intracellular carbon and energy reservoir materials.[24]

Until now, more than 150 monomer units of PHAs have been identified.[25] Notably, monomer compositions and molecular weight influence the properties of PHA polymers.[13,26] In particular, the copolymers of short-chain-length PHAs (SCL-PHAs, which contain 3 to 5 carbon atoms in their monomer units) and medium-chain-length PHAs (MCL-PHAs, which include 6 to 12 carbon atoms in their monomer units) have shown a wide range of properties, namely, from rigid to more flexible and elastic materials.[13,27] SCL homopolymers such as poly(3-hydroxybutyrate) are rigid and stiff, while MCL-PHAs are more flexible and elastic materials.[28,29] Therefore, copolymers of PHAs can be used for various industrial applications, including bulk commodities and biomedical applications such as tissue engineering, drug delivery, and vascular applications.[28,30,31] However, there has been a rising demand for PHA bioplastics in the market, and the large-scale production of PHAs is limited, representing only 1.2% of the global bioplastics production.[27] The high production costs, inconsistent polymer properties, and expensive downstream processes are the primary bottlenecks in their wide range of industrial applications.[31,32]

Due to increasing interest in PHA bioplastics, the literature studies focus on approaches to use cheap, renewable, lignocellulosic biomass to reduce PHA production costs.[10,33,34] This perspective critically reviews the recent advances and emerging technologies that may contribute to achieve sustainable, cost-competitive, and high-quality tailored PHA polymers (Figure 1). Three biomass processing units to PHA production, namely, pretreatment, fermentation, and downstream, are discussed. The following critical aspects to enable a circular bioeconomy in the PHA production process are considered:

1. The use of biomass as raw material and production costs.
2. Greener pretreatments for the sustainable extraction of cellulose, hemicellulose, and lignin from biomass to the PHA fermentation process.
3. Next-generation industrial biotechnology approaches in PHA fermentations to produce cost-competitive and high-quality PHA polymers.
4. Sustainable downstream processes to extract PHA polymers.
5. Potential scalability of this technology to real-world applications within biorefineries.

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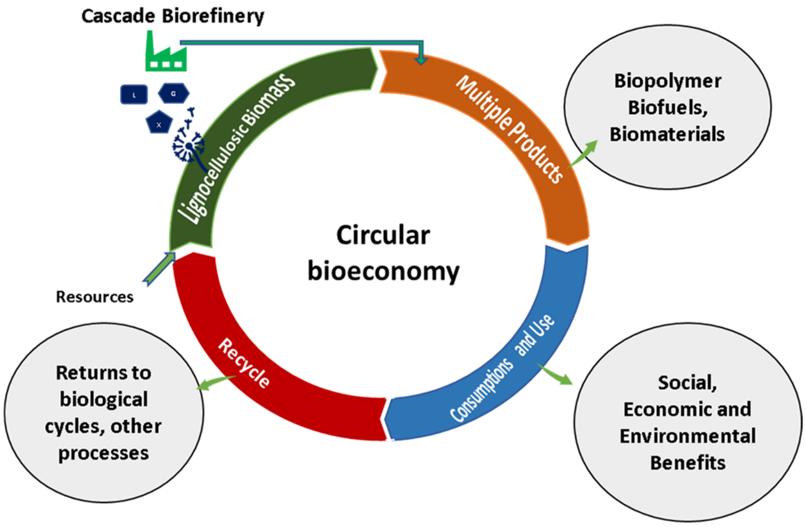

Figure 2. Conceptual circular and sustainable biomass-based biorefinery, which is fractionated into glucose (G), xylose (X), lignin (L), and further transformed into multiple value-added products. The use and consumption of these products have social, economic, and environmental benefits. After consumption, these biobased products at the end of life are sent back to biological cycles, such as nutrients to plants. The zero-waste approach in biorefinery promotes an emerging circular bioeconomy in the biorefinery concept.

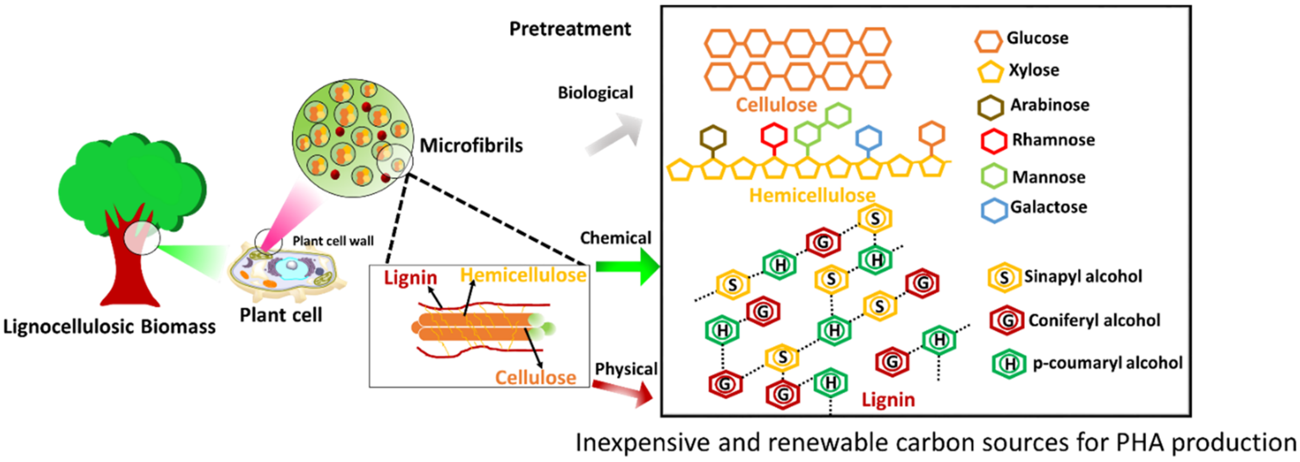

Figure 3. Schematic representation of lignocellulose and its main components: cellulose, hemicellulose, and lignin which serve as inexpensive carbon sources for microbial PHA production. Adapted with permission from ref 47. Copyright 2022, Elsevier.

■ CLOSED-LOOP BIOMASS-BASED BIOREFINERY CONCEPT

In a broad sense, a biorefinery is the processing of biomass sustainably into an array of marketable (value-added) products, biofuels, and energy.[35] To reach those targets, a biorefinery may combine various biomass conversion technologies such as chemical, thermochemical, catalytic, and biological. The long-term viabilities of biorefineries rely on the development of cascade processes which valorize cellulose, hemicellulose, and lignin in biomass, providing a diversified products portfolio with improved economic figures and zero waste emissions,[36,37] shown in Figure 2. With respect to PHA production, Dietrich et al.[33] reviewed the practicability of integration of PHA production into lignocellulose biorefineries, since this novel approach not only improves economics of PHA, but also helps valorize wastes in readily upscaled processes.[38] In the context of economic and environmental benefits, a coproduction strategy may reduce downstream costs (20%−25% of overall PHA production) and efficiently valorize the wastes.[39] Kumar and Kim[19] reviewed strategies of co-synthesis of value-added products such as amino acids, pigments, carotenoids, hydrogen, and surfactants, together with PHA production. Likewise, Koller et al.[40] showed the production of bioethanol as a coproduct along with PHA.

Feedstocks. Among various available biomasses, lignocellulose is abundant and has been assessed for multiple roles in sustainable development.[12,41] Lignocellulosic biomass includes agricultural wastes, forest residues, energy crops, and industrial wastes. Approximately 180 billion tonnes of lignocellulosic biomass is generated globally, and only 3% of it is efficiently utilized for bioeconomy products such as fuels and chemicals.[42] Lignocellulose mainly comprises three components: cellulose, hemicellulose, and lignin,[12,43,44] as shown in Figure 3. The sustainability of a lignocellulosic biorefinery depends on the depolymerizations and valorizations of cellulose, hemicellulose, and lignin.[45,46]

However, lignocellulosic biorefineries have traditionally exploited the carbohydrates fraction to generate biofuels and chemicals, while the valued lignin fraction has remained as underutilized waste.[44] Recently, advances in synthetic biology and metabolic engineering biological valorization of lignin has

Table 1. Studies Performed for PHA Production (at Laboratory Scale) Using Different Types of Lignocellulosic Biomass and Microorganisms

Biomass	Carbon source	Microorganisms	PHA	Yield g/L	ref
Rice husks	Glucose	*Cupriavidus necator*	PHB	5.0	54
Corn stover	Lignin and residual sugars	*Pseudomonas putida* KT2440	mcl-PHA	1.38	55
Rice straw	Glucose	*Ralstonia eutropha* ATCC 17699	PHB	11.42	56
Miscanthus biomass	Glucose	*Ralstonia eutropha* 5119	PHB	1.8	57
Tequila agave bagasse	Glucose	Burkholderia sacchari	PHB	11.72	58
Sugar maple	Hemicellulose	*Burkholderia cepacia* ATCC 17759	PHB	8.72	59
Orange peel	Hemicellulose	*Bacillus subtilis*	PHB	1.2	60
Hardwood	Glucose and xylose	*Paraburkholderia sacchari* IPT 101 LMG 19450	PHB	22.0	61
Corn stover	Lignin	*Pseudomonas putida* KT2440	mcl-PHA	1.5	62

become possible, as, in some microorganisms, metabolic pathways have been constructed for the conversion of heterogeneous phenolics via biological funneling to value-added products such as PHAs.[44,48−52] Utilizing lignocellulose may become a promising, greener, economic, and sustainable approach to PHA production (Table 1), as resources are not in direct competition with food values.[34,53]

■ EMERGING PRETREATMENTS TECHNIQUES FOR VALORIZATION OF LIGNOCELLULOSIC BIOMASS: PAVING THE WAY TO EFFICIENT AND ENVIRONMENTALLY FRIENDLY FRACTIONATION METHODS

An efficient pretreatment is crucial for converting lignocellulose into value-added products.[63−65] Reported life cycle and technoeconomic assessments show that the pretreatment step is energy demanding and accounts for 40% of total processing cost,[66] being the main bottleneck in the industrial-scale utilization of lignocellulose for PHA production.[33] Several pretreatment techniques such as acid, alkaline, liquid hot water, organosolv, and ionic liquids have been used to extract carbon substrates from lignocellulosic biomass.[63] Table 2 discusses these conventional methods, which typically demand high operational costs and more energy demand and generate toxic byproducts for fermentation processes, corrosion of equipment, and a challenging isolation and recovery of lignin.[66] To overcome these challenges, emerging environmentally friendly fractionation methods have been proposed, such as deep eutectic solvent-assisted strategies (DES), mechanocatalysis-assisted pretreatments, and microwave-assisted pretreatments.

Deep Eutectic Solvents (DES). Depolymerization of lignocellulosic biomass with "green solvents" has emerged as a cost-effective and sustainable solution for biorefineries.[67] Among examples in the context of green solvents, earlier ionic liquids have drawn the scientific community's attention because of their versatile properties such as negligible vapor pressure, high solvation capacity, recyclability, and low flammability.[68] Still, its industrial applications are limited due to its high cost, complex preparation process, and some toxicological issues.

These challenges have stimulated the search of new alternatives such as DES, which show similar properties to ionic liquids, but are biodegradable and more cost effective.[68,75] As in other areas, DES have attracted increasing attention as promising solvents for the lignocellulose fractionation and valorization of lignin.[76,77] DES are prepared by mixing hydrogen bond donors (HBD) such as carboxylic acid, urea, sugar, amides, etc. and hydrogen bond acceptor (HBA) counterparts such as choline chloride and their derivatives, which lead to the formation of transparent mixtures with low melting points.[75,78]

Recent comprehensive reviews on the DES-mediated lignocellulose processing have pointed out the opportunities that DES may bring for lignocellulose pretreatment and lignin isolation.[67,75,77,79] In fact, DES pretreatments have more selectivity toward lignin isolation than cellulose, which is interesting for regenerated lignin and recovered cellulose.[80,81] DES facilitate the cleavage of both hydrogen and ether bonds in carbohydrate and lignin complex structures, leading to the selective extraction of lignin.[80,82] Therefore, recently, DES have attracted increasing attention in biomass pretreatment.[83] The recycling of DES is critical for the economics of the process. Liu et al.[77] and Kim et al.[79] have evaluated the recyclability and reusability of DES, proving that DES could be recycled and reused without hampering the pretreatment performance.

Mechanocatalysis. The utilization of cellulose, a renewable feedstock, as a raw material via hydrolysis remains challenging due to its strong 3D hydrogen bond network.[84] Cellulose-to-glucose conversion is feasible via acid hydrolysis, but this chemical reaction requires harsh conditions, leading to the formation of many undesired byproducts.[85] In addition, the overall process is costly and causes corrosion to the equipment.[86] The acid-free processes, i.e., the enzyme-catalyzed glucose production from cellulose or the use of ionic liquids are excellent hydrolytic processes, yet with still tight economics.[87] Herein, solvent-free processes may become an alternative.[88] The solvent-free ball milling technology has emerged as an efficient and green alternative to conventional methods.[89] Employing the ball milling process for the depolymerization of cellulose is highly beneficial because it reduces the size of cellulose to around 15 μm, decreases the crystallinity index, and enhances the solvent-free accessibility of cellulose (bio)catalysts.[84] As a downside, due to its recalcitrant nature, cellulose ball milling requires high energy input.[90]

Interestingly, the acid catalyst used during the milling process remains, hence assisting the further chemical hydrolysis of water-soluble products at mild conditions (130 °C for 1 h) with 91% yield of glucose and 96% yield of xylose; this approach shows a promising alternative to enzymatic hydrolysis impregnation with strong mineral acids such as H_2SO_4 or HCl resulting in considerable improvement in mechanocatalysis processes[89−91] and in forming branch water-soluble oligomers with α-(1−6) linkage.[92] Schüth et al.[87] suggested that mechanical forces are beneficial in activating the "latent state" that is protonated cellobiose subunits appearing in the cellulosic chain, thus causing the requisite conformational changes for the cleavage of the glycosidic linkage, as shown in Figure 4.

Recently, the mechanocatalysis-assisted depolymerization of raw biomass to obtain low-cost metabolites for downstream applications has been comprehensively and critically re-

Table 2. Critical Discussion on Conventional Lignocellulose Pretreatment Methods

Pretreatment	Conditions	Advantages	Disadvantages	Criticism	ref
Acidic	72% w H_2SO_4, Temperature, RT, Time 30 min	Solubilized hemicellulose fraction, Simple operating process, Inexpensive	Formation of inhibitors such as organic acids, aldehydes, and phenols, Degradation of fermentable sugars, Corrosion of equipment due to use of concentrated acids	Byproducts in biomass hydrolysate might inhibit growth of microbes and reduce fermentation rates, resulting in low PHA productivity, Lack of isolation and recovery of lignin, challenging economics of the biorefinery	69
Alkaline	0.1−1.0 mol/L KOH, Temp 121 °C, Pressure 0.1 MPa	Extract partially lignin, Less degradation of fermentable sugars	Harsh pretreatment, Conditions generate toxic compounds	Low productivity of PHA due to presence of toxic compounds in hydrolysate, hence additional detoxification step is needed, which elevates the cost of the process; Process requires high-energy inputs	70
Liquid hot water	Ultrapure water, Temp 200 °C, Time 30 min	No catalyst or organic solvents are needed, Extract hemicellulose and lignin, Straightforward operational process	High energy consumption, High water consumption	High consumption of energy could increase PHA production costs and might raise sustainability issues, High consumption of water could be a major barrier to scaling up production of PHA	71
Steam explosion	0.3% w/w SO_2 impregnated on biomass, Temp 215 °C	High yield of fermentable sugars, Low impact of the process on the environment	Degradation of the hemicellulose, Generation of toxic byproducts due to harsh pretreatment conditions	Harsh pretreatment conditions produce inhibitors in thydrolysate which can negatively impact on PHA productivity, Not energy efficient	72
Organosolv	Temp 190 °C, Time 60 min, H_2SO_4 1.2% w/w, EtOH/H_2O 0.65	Extract lignin, This pretreatment is efficient for both soft and hardwood	High process costs, Lack of recycling of solvents, Concerns about solvents used in this process's safety and environmental impact	Use of this technique in PHA production might increase overall production cost of PHA, To improve the sustainability of the overall PHA process, recycling of solvents or waste is important	73
Ionic liquids (ILs)	Temp 100 °C, Time 30 min, Ionic liquid 1-H-3-methylimidazolium chloride	Mild conditions, Efficient solubilization of cellulose, Recovery of lignin may be reached, Less generation of toxic inhibitors	ILs show a negative impact on enzyme activity, ILs show poor biodegradability, Expensive process costs, Lack of recycling of ILs	ILs are a green pretreatment and produce fewer inhibitors as compared to other methods, their poor biodegradability, lack of recycling of ILs, and high process cost are major barriers to their implementation in PHA production	74

viewed.[87,93−96] The mechanocatalytic-assisted depolymerization leads to the formation of water-soluble oligosaccharide products and lignin fragments. Notably, upon further acid hydrolysis of oligosaccharides, lignin precipitates as a sulfur-free material, and lignin is separated from the solution of monosaccharides by the filtration method.[92]

Microwaves. Microwave-assisted processes are considered to be one of the promising pretreatments for lignocellulose depolymerization. They are selective, rapid, and energy efficient for thermal and nonthermal effects and directly interact with materials, accelerating physical, chemical, and biological reactions.[97,98] The emergence of the microwave as a thermal technique for pretreatment has drawn significant interest in biorefining since it is based on green chemistry principles.[99] Various studies provide mechanistic insights into the effect of microwave heating on biomass.[100−102] The microwave process offers *in situ* heating, which is superior to the conventional heating process, as volumetric heating, quick start-up, stopping, safety, and automation are advantageous. However, biomass conversion into high-yield valuable intermediate chemicals depends on the variability of some critical parameters. These parameters include (i) biomass type, (ii) size and amount of input, (iii) moisture content, (iii) reaction time, (iv) heating rate, (v) catalyst:biomass ratio, (vi) and output power.[103] Microwaves have been combined in several pretreatment conditions, such as microwave/water alone (autohydrolysis),[104] microwave/ionic liquids,[105] microwave/DES,[106] and microwave/acids[101,107] and using agricultural wastes, forest residues, energy crops, and industrial wastes. These microwave-assisted pretreatments depolymerize the biomass and sustainably isolate polysaccharides and lignin fractions for the fermentation process to produce a variety of biocommodities.

■ METABOLIC PATHWAYS FOR CONVERSION OF CELLULOSE, HEMICELLULOSE, AND LIGNIN TO SCL-PHAS AND MCL-PHAS

The metabolic pathways for PHA biosynthesis are linked to the catabolism and anabolism of bacteria and archaea.[33] PHA production starts with biomass pretreatment, which isolates polysaccharides and lignin residue, as shown in Figure 5. The fermentable sugars contained in biomass hydrolysates are transported to the cells, followed by catabolic conversion to pyruvate and ultimately to acetyl-coenzyme A (acetyl-CoA) before three subsequent steps of conversion.[108,109] The sugars are carbon and energy sources, producing adenosine triphosphate (ATP), adenosine diphosphate (ADP), nicotinamide adenine dinucleotide (NADH), or nicotinamide adenine dinucleotide phosphate (NADHP) along with the production of pyruvate.[110] The lignocellulosic hydrolysate containing sugars is metabolized by microbes using three metabolisms: Embden−Meyerhof−Parnas (EMP), Entner−Doudoroff (ED), and Pentose−Phosphate Shunt (PPS)[33] to produce PHAs. Generally, the glucose conversion pathway in bacteria is EMP. Nevertheless, the PHA-producing model bacteria such as *Cupriavidus necator* H16 do not have the gene for the key EMP enzyme phosphofructokinase. Therefore, they used the ED pathway instead of EMP.

The biological conversion of lignin is a promising solution for sustainable and cost-effective biorefineries.[44] Recently, bioconversion of lignin has been shown to hold significant potential for its valorization since some microorganisms such as *Pseudomonas putida* have evolved metabolic pathways to convert heterogeneous phenolics via a "biological funnel" to PHA

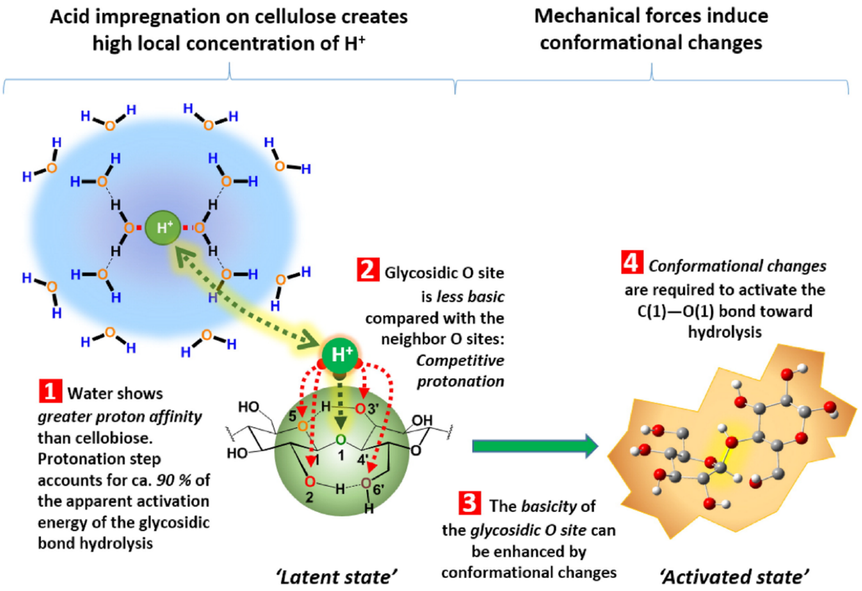

Figure 4. Mechanocatalysis aids in overcoming the difficulties associated with cellulose hydrolysis. Reprinted with permission from ref 87. Copyright 2014, Elsevier.

bioplastics.[49,50,111,112] Until now, several catalytic and enzymatic methods have been investigated for the depolymerization of recalcitrant lignin.[113−115] Among them, lignin-first approaches have received more attention because they operate at mild conditions and give uniform and few products.[116] Abu-Omar et al.[117] defined lignin-first as an active stabilization method that releases lignin from plant cells and suppresses the condensation reaction via catalysis and protection group chemistry. It is an integrated approach that produces value from both lignin and polysaccharides and aims to achieve atom-efficient and maximum utilization of the lignocellulosic biomass in a sustainable manner. In the lignin first approach, three steps are involved: (i) extraction of lignin from whole biomass either by using organic solvents (organosolv pretreatment) or acid catalyzed reaction, (ii) stabilization of intermediates produced in order to prevent condensation of reactive species generated in lignin depolymerization, and (iii) subsequently, depolymerization if it is not fully stabilized at the stabilization step. The lignin first approach converts lignin into its phenolic monomers such as *p*-coumaric acid, caffeic acid, ferulic acid, and sinapinic acid, which can be utilized by various bacteria strains such as *Oceanimonas doudoroff*,[118] *Cupriavidus basilensis* B-8,[49] and *Pseudomonas putida* KT2440[44] to produce PHAs. These ligninolytic bacteria use the lignin phenolic derivatives via biological funneling to yield protocatechuate or catechol. These intermediate precursors can further be catabolized through the *β*-ketoadipate pathway to produce acetyl-CoA and assist the synthesis of PHAs, shown in Figure 5b.

■ EMERGING FERMENTATION STRATEGIES

The success of industrial-scale PHA fermentations using lignocellulose is mainly dependent on four critical factors (i) cost-competitive PHA production, (ii) efficient utilization of lignocellulosic feedstock, (iii) high quality of PHA production, and (iv) green and environmentally friendly fermentation process for PHA production.[31,119−121] While the scientific community has been devoting its efforts to large-scale PHA production for over three decades, the high productivity and superior quality of PHA polyester are two critical factors determining its market applications.[122] Conventional fermentation strategies such as discontinuous fermentation processes lead to the high production costs of PHAs. The high production of PHAs is mainly due to the necessity of sterilization on each fermentation step, high freshwater, and energy demand.[123] Additionally, conventional fermentation processes show limited control on monomer compositions, which results in the formation of poor quality PHAs.[124,125] Thus, implementing an appropriate fermentation strategy can boost the bioconversion of lignocellulosic biomass wastes to achieve cost-effective production of PHAs. In the current emerging trend, kinetics studies, contamination-resistant strains such as extremophiles, nonsterile open-stage continuous fermentation, and mixed microbial consortia (MMC) have been reported to enhance PHA productivity production of tailored PHA polymers and cost-effective as well as sustainable PHA production. In Table 3, emerging fermentation strategies are shown, with the aim to improve PHA production in terms of cost and environmentally friendly processes and approaches to achieving them.

Two halophiles, such as *Halomonas bluephagenesis* and *Halomonas campaniensis*, have been successfully used as platform strains for next-generation industrial biotechnology (NGIB) due to their versatile properties, such as the ability to grow rapidly in open and continuous fermentation processes.[121] Further, to improve the performances of strains, several molecular engineering tools such as CRISPRi (clustered regularly interspaced palindromic repeats)and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated with protein 9) and morphology engineering tools such as increasing oxygen availability and promoter engineering for enhancing PHA accumulations have been developed and successfully incorporated into *Halomonas* spp.[126,127] The *Halomonas* spp. has been utilized as a suitable NGIB platform for the production PHAs and their other coproducts such as proteins, biosurfactants, amino acids, ectoines, etc.[128] Recently, an engineered *Halomonas bluephagenesis* TDHCDR383 strain has been exploited for PHA production under continuous fermentation conditions. It reaches more than 90 g/L cell dry weight (CDW), corresponding to 79% of PHAs.[129] In another

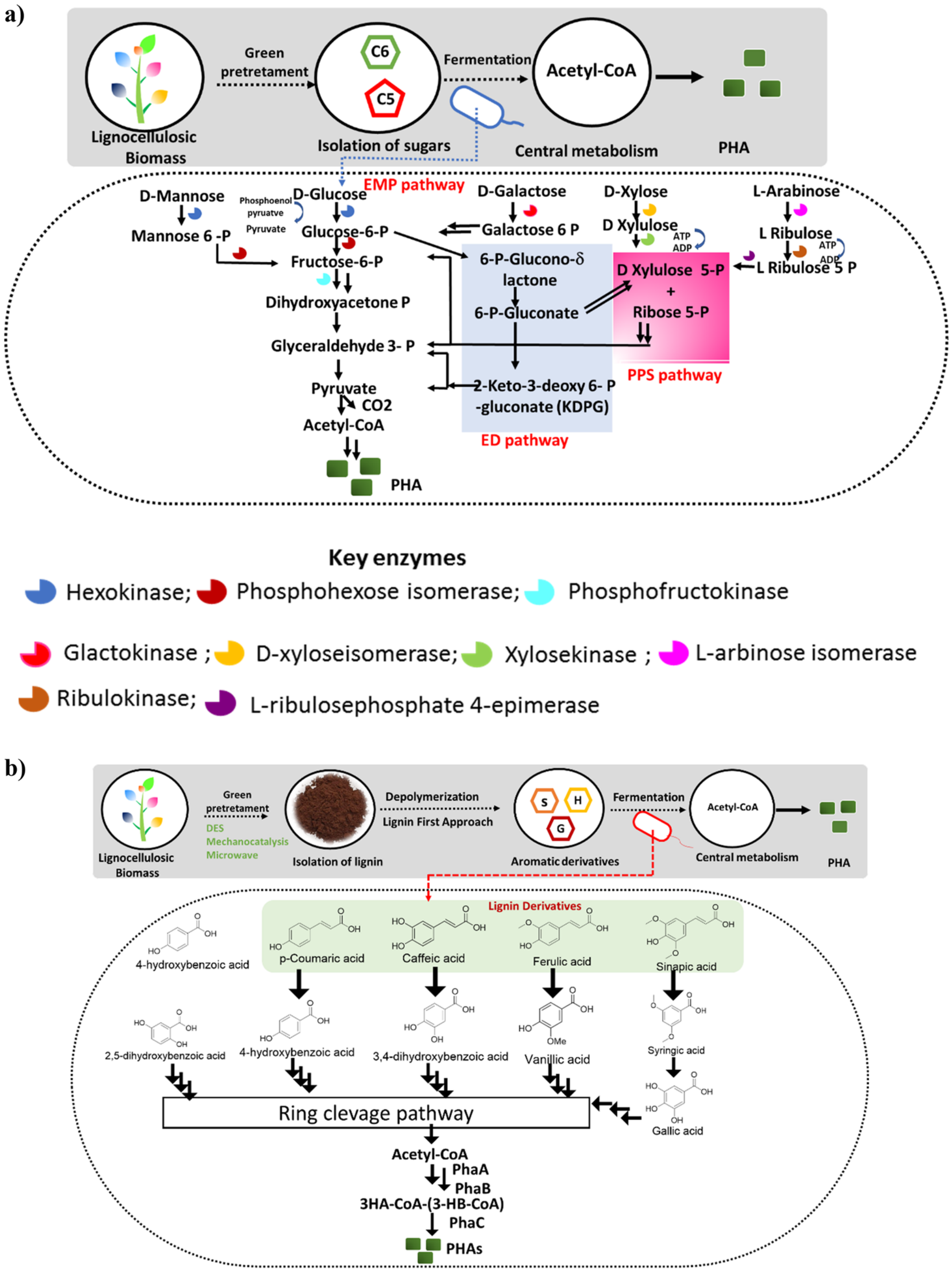

Figure 5. Metabolic pathways of conversion of fermentable sugars and lignin from lignocellulosic biomass: (a) metabolic pathway of fermentable sugars to PHAs, (b) metabolic pathway to lignin to PHAs: PhaA, β-ketothiolase; PhaB, acetoacetyl-CoA reductase; and PhaC, PHA synthase. Adapted with permission from refs 33, 47, 118: (a) Copyrights 2019 and 2022, Elsevier. (b) Copyright 2015, American Chemical Society.

study, a *Halomonas bluephagenesis* TD40 strain was used, which even reached 100 g/L cell dry weight (CDW) comprising 60% of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P3HB4HB) in a 5m^3 fermenter.[130]

The production costs of PHAs decrease with technological advancements, such as using second-generation feedstock as the raw material and next-generation industrial biotechnology approaches. In 1990, the costs of PHAs were 15−16 USD per kg of PHA; currently, it is 1.5−2.5 USD per kg, which is still higher than conventional plastics. However, to develop the PHA process based upon lignocellulosic biomass, relevant testing on the pilot scale after optimization of the process on the laboratory scale is necessary. So far, only a few examples of PHAs on a pilot scale have been reported;[61,131] these results provide confidence in the persuasion of this scheme for large-scale PHA production.[123] Furthermore, high cell density cultivation, cell recycling, and model-based footprint area analysis are recommended for pilot scale and industrial scale PHA productions as they increase PHA accumulation, especially with low culture volume, reduce wastewater generation, and improve its economics.[132,133] Therefore, adapting NGIB approaches and appropriate cultivation techniques will help resolve the challenges during the scaling up of PHA production using lignocellulosic biomass as a feedstock.

Table 3. Emerging Fermentation Strategies to Achieve Cost-Effective, High-Quality, and Sustainable PHA Production

Strategies	Aim	Approaches to achieving it	ref
Open and continuous fermentation	Reduce energy input, Easy operation of fermentation process, Improve process effectiveness	Develop extremophile microbes that can survive in high or low temperature, pH, pressure environments and high salinity	31, 119, 120, 130
High-density cell cultivation	Improve PHAs productivity, Cell recycling and cell retention, Reduce PHA cost	Two-time carbon and nitrogen feeding strategy, Manipulate mechanisms of quorum sensing and oxygen uptake via genetic engineering tool	134−137
Genome editing	Improve conversion of aromatic compounds	CRISPR/Cas9n-λ-Red	138
Sea water	Sustainability, Reduce PHA cost	Develop and examine extreme halophilic bacteria	139
Ceramic and cement bioreactor	Reduced capital investment	Develop and examine contamination-resistant strains	140
Kinetics	No error in optimization, Less time for optimization	Luedeking−Piret model, Verhulst model, Mathematic modeling	133, 141

COST-EFFECTIVE AND SUSTAINABLE STRATEGIES FOR RECOVERY OF PHAS

The extraction of PHA polyesters from inside the cells of the microorganisms is challenging. It is the main bottleneck in developing an economically feasible PHA fermentation process because it is an intracellular product and is less soluble in eco-friendly solvents.[32,142] There are two ways to separate PHAs from microbial biomass: dissolved noncellular PHA mass (NPCM) via chemical or mechanical digestion or extraction of PHAs with chemical solvents, which alter the permeability of the cell membrane and dissolve PHA polyester.[143] In general, the non-PHA parts of the biomass are dissolved into halogenated solvents such as chloroform, dichloromethane, sodium hypochlorite, etc.[144] Although extracting PHAs using halogenated solvents is a simple and more straightforward method and gives a high recovery yield, purity of polymer, and molecular weight, this technique is expensive and increases the environmental burden due to the use of toxic solvents.[133]

The downstream process is a valuable part of the overall PHA production process; hence, the efficient and sustainable extraction of PHA polyester is crucial to the realization of the circular bioeconomy in the overall PHA production process.[145]

Recently, the scientific community has investigated several nonhalogenated solvents, such as ketones, lactones, carbonate esters, alcohols, etc., for PHA extraction as a green alternative to halogenated methods. Koller et al.[146] developed a novel approach to extracting SCL-PHAs from microbial biomass using SCL-PHA antisolvent acetone at elevated temperature and pressure. When the authors compared the results of the new and conventional extraction processes, they found that the new method shows a similar performance in terms of recovery (98.4% vs 97.7%), and the purity of PHAs of 97% is identical in both cases. The extraction of PHAs with acetone was rapid (20 min) compared to the traditional method (12 h). The recovery of acetone after extraction of PHAs is attributed to the sustainability of the process. Samorì et al.[147] used dimethyl carbonate and a switchable anionic to extract PHAs from microbial biomass. They recovered more than 85% of PHAs with 95% purity of the product by removing PHAs with dimethyl carbonate, while 99% of recovery and 90% of PHA purity were obtained by extracting PHAs with a switchable anionic surfactants. Arikawa et al.[148] demonstrated the simple and rapid method by using sodium dodecyl sulfate (SDS) sonication treatment for removing PHAs from bacterial cells. The authors reported purity of 96% of the recovered PHAs. Dubey et al.[17] showed an eco-friendly approach toward PHA extraction of intracellular PHAs substituting halogenated solvent by ionic liquid (IL) 1-ethyl-3-methylimidazolium diethyl phosphate ($[C_2mim]$-$[(C_2)_2OPO_3]$) as an extractant. The authors could recover 60% of PHAs from microbial biomass. The authors used ILs for two successive cycles to solubilize the microbial biomass, thus reducing the cost of the downstream process.

Mechanical disruption has received much attention as a solvent-free and green approach to PHA extraction. This method uses mechanical forces to break the cell walls of the bacteria. The well-known mechanical disruption technique is high-pressure homogenization (HPH), in which microbial biomass is pushed into the chamber and flows with high pressure via a narrow cavity subjected to higher shear stress which causes cell disruption.[149] However, this technique is environmentally friendly for extracting PHA polymers from the cell walls of bacteria; it requires an additional investment such as filtering equipment which increases the cost of the downstream process of PHAs.

Supercritical fluid (sCO_2) has been investigated for efficient extraction of high value-added products such as PHA biopolymer from various natural matrices.[142,149] The advantage of using supercritical fluids is that they have excellent solvation power for liquids and gas. For this reason, sCO_2 diffuses through solid and dissolved materials. sCO_2 was investigated for extraction of SCL-PHA and MCL-PHA polymers from biomass of the different types of bacteria strains.[150] Hejazi et al.[151] used the Taguchi statistical model to optimized sCO_2 parameters such as temperature, pressure, volume of methanol as a modifier, and cell history on the disruption efficiency for extraction of PHA polymers from biomass of *Ralstonia eutropha*. The authors could achieve 90% recovery of PHA polymers at optimum conditions, i.e., temperature of 40 °C, pressure of 200 atm, and 0.2 mL of methanol. The authors claim that sCO_2 extraction is comparable to other recovery methods; in addition, this recovery method is environmentally friendly. These findings are opposite to the conclusions made by the other researchers; according to them, sCO_2 is a more efficient solvent for lipid and other hydrophobic materials, but not for PHAs.[149] Koller's[150] experimental study demonstrates that sCO_2 is a feasible and promising method for decreasing PHA rich biomass, but is feasible for PHA polymer recovery.

Hypotonic disintegration is known as hypoosmotic disruption, which is used to extract PHA polymers from biomass of extremely halophilic archaea. Notably, under the influence of a hypotonic environment, the halophiles disrupt their cells without use of any organic solvent. For a more detailed study of hypotonic lysis, the readers are advised to refer to the review of Koller[150] for established and advanced approaches to the recovery of PHAs from microbial biomass.

Recently, aqueous two-phase systems (ATPS) have attracted increasing attention from the scientific community and

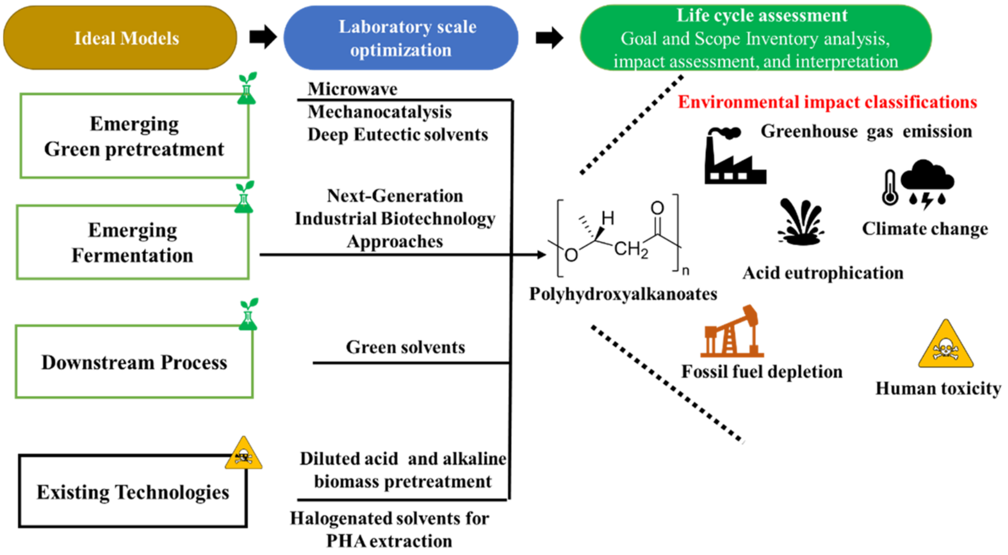

Figure 6. Schematic cradle to gate representation of emerging biomass pretreatment, emerging fermentation, and downstream strategies to PHA production. Life cycle assessment can potentially drive the development of emerging technologies with an improved ecological performance by identifying ecological hotspots and assessing existing technologies.

industrialists because ATPS comprise higher aqueous content of around 70%−80%; therefore, they provide eco-friendly separation of complex biomolecules.

Additionally, the materials that form two layers of ATPS are often safe and environmentally friendly compared to conventional solvent extraction methods. The materials used in the ATPS system can be polymer−polymer or polymer−salts.[143] Leong et al.[152] performed PHA extraction using thermoseparating polymers such as an ethylene oxide−propylene oxide copolymer in ATPS as the primary purification step. With this proposed strategy, the authors could achieve a yield of 98% of PHAs under optimized conditions.

Besides solvent extractions, several digestion techniques have been used to solubilize noncellular biomass. Broadly, digestion techniques are classified into three types: chemical, enzymatic, and biological. Chemical digestion methods utilize chemical compounds such as sodium hydroxide (NaOH), sodium hypochlorite (NaClO), and sodium dodecyl sulfate (SDS) to break the cells of bacteria and extract intracellular PHA polymers.[29] It is a promising option in PHA recovery, as chemical digestion attributes economic and environmental advantages over conventional PHA extraction methods. The economic benefits include recycling regents, using wet biomass, and saving reagent costs. The main drawback of chemical digestion is polymer degradation,[153] but further process optimization can resolve this problem. Technoeconomic and life cycle assessment studies require evaluating its feasibility for large-scale PHA extraction. Alternatively, the enzymatic digestion approach is more eco-friendly. This approach uses crude or pure enzymes to disrupt the bacteria cells and extract PHAs. The main advantages of enzymatic digestion are the low energy input and aqueous recovery, which give excellent yield and purity of the product. However, the high costs associated with enzymes are the main barrier to scaling up this method. Kachrimanidou et al.[154] developed the novel enzyme-based digestion approach to extract PHAs. In this approach, the authors used crude enzymes, i.e., *Aspergillus awamori* obtained from solid-state fermentation. This approach facilitated excellent recovery (98%) and purity (97%) without adding chemical solvents.

In contrast to the enzymatic digestion approach, biological digestion uses viruses, bacteria, and mealworms to release PHA granules from the bacteria cell walls. Biological digestion will be a promising alternative for non-PHA cell mass (NPCM) digestion methods since it does not require expensive instruments. However, the digestion process takes a long time and needs additional posttreatment.

Conclusively, developing a sustainable and cost-effective downstream processing is crucial to scaling up PHA production. The eco-friendly and green downstream technologies are in high demand for industrial-scale PHA production as the world heads toward a circular and sustainable bioeconomy. Furthermore, the life cycle assessment study for the selection of a sustainable downstream process for PHA polymer extraction is crucial.

■ SUSTAINABILITY ASSESSMENT OF UPSTREAM AND DOWNSTREAM PROCESSES OF PHAS

Since lignocellulosic biomass to PHA bioplastics is an emerging technology, producing this biopolymer at the industrial scale faces various challenges such as technology development in biomass conversion, optimization of fermentation, and a downstream process to make PHA production cost competitive and sustainable compared to other conventional alternatives. Additionally, all stages of biomass processing and the end-life phases of PHAs should be evaluated to check the environmental benefits compared to their conventional counterpart, such as fossil-based plastics. When discussing new technologies or products, the funding agencies ask the researchers how the product is environmentally beneficial. How much will energy and greenhouse gas emissions be reduced?[155] The life cycle assessment (LCA) is the most advanced tool that can boost the development of emerging technologies with an improved ecological performance by identifying environmental hot spots and comparing current conventional alternatives,[156] as shown in Figure 6.

Life Cycle Assessment (LCA) of Upstream Process. Feedstock selection and its couse for energy production can significantly improve the PHA's environmental performance and make PHA-based material have net zero carbon emissions.[14]

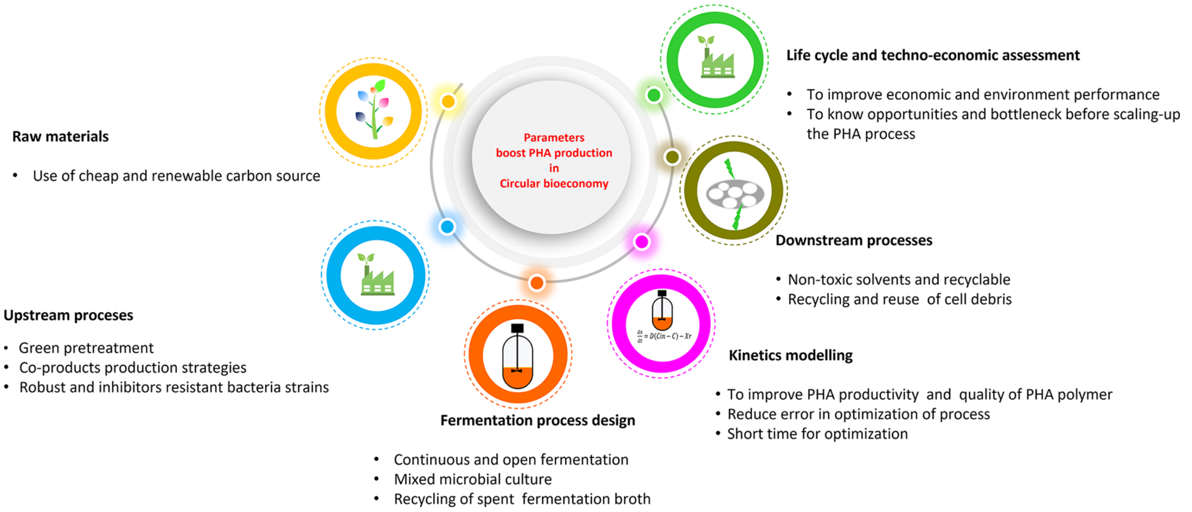

Figure 7. Schematic representation of biomass conversion processes and their connections among them that boost PHA production into the concept of a circular bioeconomy.

Kachrimanidou et al.[157] employed a "cradle-to-gate" LCA approach to assess the ecological impact of PHB production using biodiesel industry byproducts as the carbon substrate. The LCA study showed that PHB production using biodiesel industry byproducts emitted greenhouse gas emissions of ca. 0.64 kg CO_2-eq/kg PHB and abiotic depletion potential of 61.7 MJ/kg PHB, which were significantly lower than fossil-based propylene (greenhouse gas emission of 2.0 kg CO_2-eq/kg and abiotic depletion of 73.35 MJ/kg). These results represent that using biodiesel industry byproducts leads to the sustainable production of PHB. In another study, Koller et al.[158] used the sustainable process index (SPI) methodology to assess ecological sustainability of the process development for microbial production of PHA by using industrial surplus material whey. When authors compared SPI data of whey-based PHA production with its fossil-based counterparts, they found that whey-based PHA production has a higher ecological impact than its fossil-based counterparts. The reason for this higher ecological impact is the higher consumption of electricity in the fermentation step due to its long time (100 h). The authors discovered an intriguing factor after further examination of SPI data. The ecological impact of whey-based PHA production is reliant on the ecological pressure induced by electricity provisions. In this study, the calculation of SPI data has been carried out with the EU 27 mix technologies which leading to high ecological impact due to use of fossil energy and nuclear energy in the European power station. Further, the authors suggest that the use of green electricity for this process will make whey-based PHA production more sustainable.

Kookos et al.[159] performed LCA to investigate the environmental impact of PHB production via the microbial fermentation process using soybean oil, corn sugar, and sugar cane as inexpensive carbon substrates. The authors compared the greenhouse gas emissions (GHG), nonrenewable energy use (NREU), acidification potential (AP), and eutrophication potential (EP) associated with PHA production to those of its fossil-based counterparts, such as high density polyethylene (HDPE) and polypropylene. The results of their LCA study reveal that the choice of raw materials and the energy allocation methodology have significant impacts on GHG, NREU, AP, and EP. The appropriate choices of the raw material (in this case soybean oil) and energy allocation methodology must significantly reduce GHG (−2.58 kg CO_2-eq/kg of PHB) and NREU (−28.4 MJ/kg polymer) values for PHA production compared to those values obtained for fossil-based HDPE GHG (1.8 kg CO_2-eq/kg of polymer) and NREU (79.39 MJ/kg of polymer). Thus, PHA production is more beneficial over fossil-based plastics.

Recently, Vogli et al.[160] performed "cradle-to-grave" LCA to evaluate the environmental impact of PHA production via a hybrid thermochemical−biological process by using anaerobic sludge as an inexpensive carbon source. In this study, the system boundaries included (i) sludge pyrolysis with subsequent production of volatile fatty acids, (ii) PHA-enriched production by using a mix microbial culture (MMC), (iii) PHA extraction from microbial biomass, and (iv) PHA end of life. The data of their LCA study show that PHA production using sludge as the feedstock has comparable environmental performances with those of petroleum-based polymers in terms of nonabiotic greenhouse gas emissions and energy demand.

LCA of Downstream Process. The downstream process of PHAs is challenging, as stated above. Hence, it is a significant bottleneck in implementing the PHA process for large-scale production.[14] Therefore, an LCA study should be performed before the commercialization of PHAs. Fernández-Dacosta et al.[161] evaluated three downstream processing methods based on alkali, surfactant−hypochlorite, and solvent treatments. The LCA study revealed that the alkali method is more sustainable than the other two methods since the alkali method produced a global warming potential of 2.4 kg CO_2-eq/kg PHB and nonrenewable energy use of 106 MJ/kg PHB. The solvent extraction has increased an environmental burden (global warming potential of 4.30 kg CO_2-eq/kg PHB and NREU of 156 MJ/kg PHB).

ATPE is an advanced technique of PHA extraction that utilizes thermoseparating polymers such as the ethylene oxide−propylene oxide copolymer (EOPO). Leong et al.[162] evaluated the environmental performance for surfactant−hypochlorite digestion based on with and without thermoseparating ATPE. The result of the LCA study showed that PHA extraction with thermoseparating ATPE is environmentally friendly and cost effective since it reduces chemical consumption, and the EOPO polymer is recyclable.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

Various low-cost lignocellulosic biomass materials have been exploited for PHA production. However, bioconversion of lignocellulosic biomass to PHA is still under development at the laboratory scale. Their implementation at the pilot scale is still facing several challenges and limitations, but it gives a new milestone for scientists to address these challenges in future research. Using advanced pretreatment techniques such as microwave, mechanocatalysis, and deep eutectic solvents to extract sustainable, low-cost metabolites for PHA production in the biorefinery addresses economic, environmental, social, and engineering issues sustainably. These techniques are still in the development phase. An integrated biorefinery approach is needed in the PHA production process to produce some other value-added coproducts. This approach helps reduce PHAs' costs and valorize waste at an upscale process[19,33] as shown in Figure 7.

The significant bottlenecks of fermentation are the low productivity and inconsistent polymer properties of PHAs from the lignocellulosic hydrolysate.[27] Recently, the modification of PHA-producing strains using synthetic biology has been widely studied. Furthermore, a strain's improvement should focus on two areas: (i) efficient utilization of various lignocellulosic hydrolysates to achieve higher accumulation and lower by-product generation and (ii) development of strains that can use inhibitors such as acetic acid and formic acid to produce PHAs. However, adopting the physiological and kinetic models in fermentation is necessary to optimize the productivity and quality of the PHA polymer.

Unlike other biopolymers, PHAs accumulate inside the cells of prokaryotes. Hence, PHAs' downstream processes are complex and costly. Several studies were conducted to make it easy and sustainable. In this regard, synthetic biology tools can be used to change the sizes and shapes of bacteria cells.[52] However, the small sizes of the cells make downstream processes complicated and costly. In contrast, more significant bacteria accumulate more PHAs and promote gravity separation of cells from the broth.

CONCLUSIONS

The circular bioeconomy model emphasizes social, economic, and environmental benefits; PHA production using lignocellulosic biomass perfectly fits into the emerging circular bioeconomy concept. Greener biomass pretreatment, next-generation industrial biotechnology-based fermentation strategies, and sustainable downstream strategies are crucial to recycled and reused wastes that boost PHA production into a circular bioeconomy concept. The valorization of lignocellulosic sugars and lignin derivatives into SCL-PHA and MCL-PHA is discussed. Converting cellulose, hemicellulose, and lignin into PHA via microbial fermentation will be highly beneficial to moving PHA production closer to sustainability. A high potential remains, but many aspects must be convincingly developed and substantiated for a full circular bioeconomy in PHA production.

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Notes

The authors declare no competing financial interest.

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Richard Ahorsu is currently a postdoctoral scientist and grant coordinator at Universitat Rovira i Virgili, Tarragona, Spain. He received a Ph.D. degree in nanoscience, materials, and chemical engineering from Universitat Rovira i Virgili in 2021. He pursued his M.Sc., Eng., and Ph.D. from Universitat Rovira i Virgili under the guidance of Professor Francesc Medina and Dr. Magda Constantí. He joined Dr. Roberto Rinaldi's "Tomorrow's Chemical Technologies Lab" at Imperial College London as a visiting doctoral student in 2019. He specializes in green chemistry, heterogeneous catalysis, and fermentation (biocatalysis).

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James Winterburn received his Ph.D. at University of Manchester in 2011 in the area of biosurfactant production, supervised by Dr. Peter Martin. In 2013, he joined the Department of Chemical Engineering and Analytical Science as a lecturer and was promoted to senior lecturer in 2018 and to reader in 2021. His current research interests are focused on industrial biotechnology, fermentation processes, and the efficient production and separation of biobased products, including biosurfactants and biopolymers. He is also programme director for the M.Eng. with Industrial Experience degree program. James is a co-founder of Holfierm Limited, spun-out from his research group in 2018. Holiferm is commercializing biosurfactant production technology and currently produce sophorolipid biosurfactants at the pilot scale. Prior to his lectureship, James worked as a postdoctoral research associate in the Multiphase Processing Group on an EPSRC funded Challenging Engineering Project.

Professor Francesc Medina graduated in chemical sciences from the University of Barcelona (1981); he did his doctoral thesis under the supervision of professors Jesús Eduardo Sueiras and Pilar Salagre at the URV (1993). In 2009, he received the ICREA Acadèmia Award from the Generalitat de Catalunya, and in 2014, he was appointed distinguished professor at the URV. He has also participated as principal investigator in more than 100 competitive projects and in more than 120 research and technology transfer projects with companies. He directs the Group de Catàlisi Heterogènia of the URV (CATHETER) and the consolidated research group of the Generalitat MEtA (materials engineering and their applications). He is also a member of the EMAS group of the URV (engineering of materials and micro/nanosystems). He cofounded the technology spin-off company APLICAT, SL, of which he is the scientific director. He participates in the URV and has the purpose of commercializing the technologies created in the AMIC innovation center. In addition, he has also built water purification plants in our territory.

Magda Constantí holds bachelor's degree in biology from University of Barcelona in 1984; then, she did her master's degree in genetics in 1985. Her Ph.D. focused on the biodegradation of organic sulphur contained in coal, in 1992. Afterwards, she did several postdoctoral stays at the University of California, Los Angeles (USA), under the supervision of Dr. Robert P. Gunsalus. The study was about the genetic regulation of different metabolic processes in *E. coli*. She also performed several studies on the microbiology of alcoholic and malolactic fermentations at the Enology Faculty at the University Rovira i Virgili. Currently, she is an associate professor at the Chemical Engineering Department, and her research focuses on the valorization of biomass for the production of lactic acid and PHB, as well as the biodegradation of xenobiotic compounds.

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