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Integrated Biorefinery Design with Techno-Economic and Life Cycle Assessment Tools in Polyhydroxyalkanoates Processing

Vaibhav Vilas Andhalkar, Shin Ying Foong, Seng Hon Kee, Su Shiung Lam, Yi Herng Chan, Ridha Djellabi, Kesaven Bhubalan, Francesc Medina, and Magdalena Constantí**

To support and move toward a sustainable bioeconomy, the production of polyhydroxyalkanoates (PHAs) using renewable biomass has acquired more attention. However, expensive biomass pretreatment and low yield of PHAs pose significant disadvantages in its large-scale production. To overcome such limitations, the most recent advances in metabolic engineering strategies used to develop high-performance strains that are leading to a new manufacturing concept converting biomass to PHAs with co-products such as amino acids, proteins, biohydrogen, biosurfactants, and various fine chemicals are critically summarized. This review article presents a comprehensive roadmap that highlights the integrated biorefinery strategies, lifecycle analysis, and techno-economic assessment for sustainable and economic PHAs production. Finally, current and future challenges that must be addressed to transfer this technology to real-world applications are reviewed.

1. Introduction

Plastics produced from fossil resources through petroleum refinery processes have played a key role in modern society over the past decades. However, our heavy dependency on fossil resources and derived products has led to serious issues such as extreme climate change, global warming, increasing the carbon-footprints, and the rapid depletion of fossil resources, which pose a threat not just to humans but also to the entire planet.[1–4] To cope with these global challenges, there is an urgent need to substitute fossil-based plastics with environment-friendly and bio-based plastics from renewable resources.[5] Among various available bioplastics, polyhydroxyalkanoates (PHAs) have received more attention in both academic and industrial research fields because of their biodegradability in soil and marine environment at ambient conditions,[6,7] and have shown great potential as a green replacement for fossil-based plastics.[8–10] Notably, emerging PHAs bioplastics with green features are continued to make incursions into the commercial market, which is expected to be worth USD 28 billion tons by 2025.[11] The current market volume occupied by PHAs is 23 000MT, at a compound annual growth rate of 6.28% from the

V. V. Andhalkar, R. Djellabi, F. Medina, M. Constantí
Department d'Enginyeria Química
Universitat Rovira i Virgili
Tarragona 43007, Spain
E-mail: magdalena.constanti@urv.cat
S. Y. Foong, S. S. Lam
Higher Institution Centre of Excellence (HICoE)
Institute of Tropical Aquaculture and Fisheries (AKUATROP)
Universiti Malaysia Terengganu
Kuala Nerus, Terengganu 21030, Malaysia
E-mail: lam@umt.edu.my
S. H. Kee, K. Bhubalan
Faculty of Science and Marine Environment
Universiti Malaysia Terengganu
Kuala Nerus 21030, Malaysia
S. S. Lam
Center for Transdisciplinary Research
Saveetha Institute of Medical and Technical Sciences
Saveetha University
Chennai, Tamil Nadu 602105, India
S. S. Lam
University Centre for Research and Development
Department of Chemistry
Chandigarh University
Gharuan, Mohali, Punjab 140413, India
Y. H. Chan
PETRONAS Research Sdn. Bhd. (PRSB)
Lot 3288 & 3289, off Jalan Ayer Itam, Kawasan Institusi Bangi, Kajang, Selangor 43000, Malaysia

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mame.202300100

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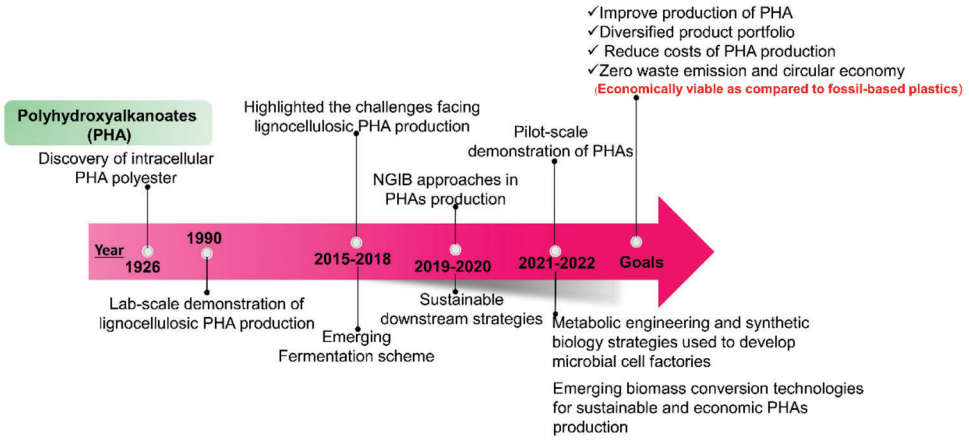

Figure 1. Historical milestones and scientific impact of the development of sustainable and economic PHAs production using lignocellulosic biomass as the raw material.

last 4 years.[12] The Food and Drug Administration of the United States (US-FDA) has approved permission to the use of PHAs bioplastics for food contact and biomedical applications because of their biocompatibility and nontoxic nature.[13,14] In addition, the demand for PHAs is gradually increasing in various industrial applications.

Although PHAs are environment-friendly and biocompatible polyesters, their high selling price (4 to 5 € kg^{-1}) in comparison to fossil-based plastics (less than 1 € kg^{-1}) limits their competition with fossil-based plastics in the commercial market or their broader applications as commodity plastics.[15–17] The high selling price of PHAs is associated with the use of expensive carbon substrates, which accounted for 50% of its overall costs of production. To overcome such a challenge, intensify research efforts have focused on the increased utilization of renewable and inexpensive carbon sources for PHAs production.[18] In this context, the biotransformation of lignocellulosic biomass to PHAs has acquired immense attention in both academic and industrial sectors because these raw materials are abundant, carbon neutral, nonedible, and low-cost carbon sources.[19]

The designing of the biorefinery process for PHAs production using lignocellulosic biomass has mainly been governed by advances in biomass pretreatment technologies, metabolic engineering, synthetic biology tool, and strategies to produce high-performance strains, fermentation processes, and downstream technologies. A chronological summary of the evolution of PHAs production using biomass wastes is shown in **Figure 1** to illustrate the historical milestones and scientific impact in the development of sustainable and economic biorefinery routes for PHA production. For the first time in the 1990, Bertrand et al. investigated lab-scale PHAs production with *Pseudomonas pseudoflava* by using renewable biomass waste as a carbon substrate. Despite biomass potential as a promising raw material, the low yield of PHAs is the major bottleneck in the utilization of lignocellulosic-based substrate for large-scale PHAs production. The challenges in the utilization of lignocellulosic substrates for PHAs have not been demonstrated until two decades, later, in the 2015 and 2018, Obruca,[20] Wang et al.,[21] and Rodriguez-Perez et al.[22] reviewed challenges in scaling up microbial PHAs process using renewable feedstock. These authors highlighted that the recalcitrant nature of lignocellulosic biomass represents the technical challenges in obtaining fermentable sugars from renewable biomass, and it is a major barrier to its use as feedstock in the biorefinery plant. Furthermore, Koller[23] highlights conventional fermentation setups based on the batch, fed-batch, or cyclic-fed batch often leading to low productivity and showing inadequate control of the polymer compositions. However, significant development has been made in pretreatment and fermentation technologies, still, most of the technologies are at lab-scale.

Besides upstream strategies, downstream processing of PHAs is more complicated and expensive. Conventional downstream strategies use a large number of halogenated solvents to extract PHAs polyesters from microbial biomass, compromising the green and sustainable features of the bioplastics and accounting for 20% to 25% of the overall costs of PHAs production.[24,25] From the 2019 to the 2020, the researcher explored various green and sustainable downstream strategies;[12] nevertheless, despite improvements in downstream strategies, the purity and yield of polymers obtained from microbial biomass are lower than conventional downstream approaches. To circumvent all the above challenges, the researchers focus their attention on finding promising alternatives to current industrial biotechnology (CIB) approaches used in PHAs production. In this context, next-generation industrial biotechnology (NGIB) in PHAs production has attracted increasing attention as an innovative and emerging approach. NGIB approach in PHAs production expands new opportunities for making bioprocess for PHAs production economically more viable and sustainable.[26] One of the great achievements of NGIB is the *Halomonas* spp. successfully engineered to produce multiple products along with PHAs in open and continuous processes. In 2021, Matos et al.[27] showed a demonstration of pilot-scale PHAs production with mixed microbial culture (MMC) by using renewable feedstock. Furthermore, the emerging consolidated bioprocesses approaches and advancement in metabolic engineering and synthetic tools in gene editing, construction of metabolic pathways, and enzyme engineering set the scientific community on the path toward the design of integrated lignocellulosic biorefinery approach for PHAs production.[18,28–30] While designing an integrated lignocellulosic approach, the whole biorefinery process of PHAs

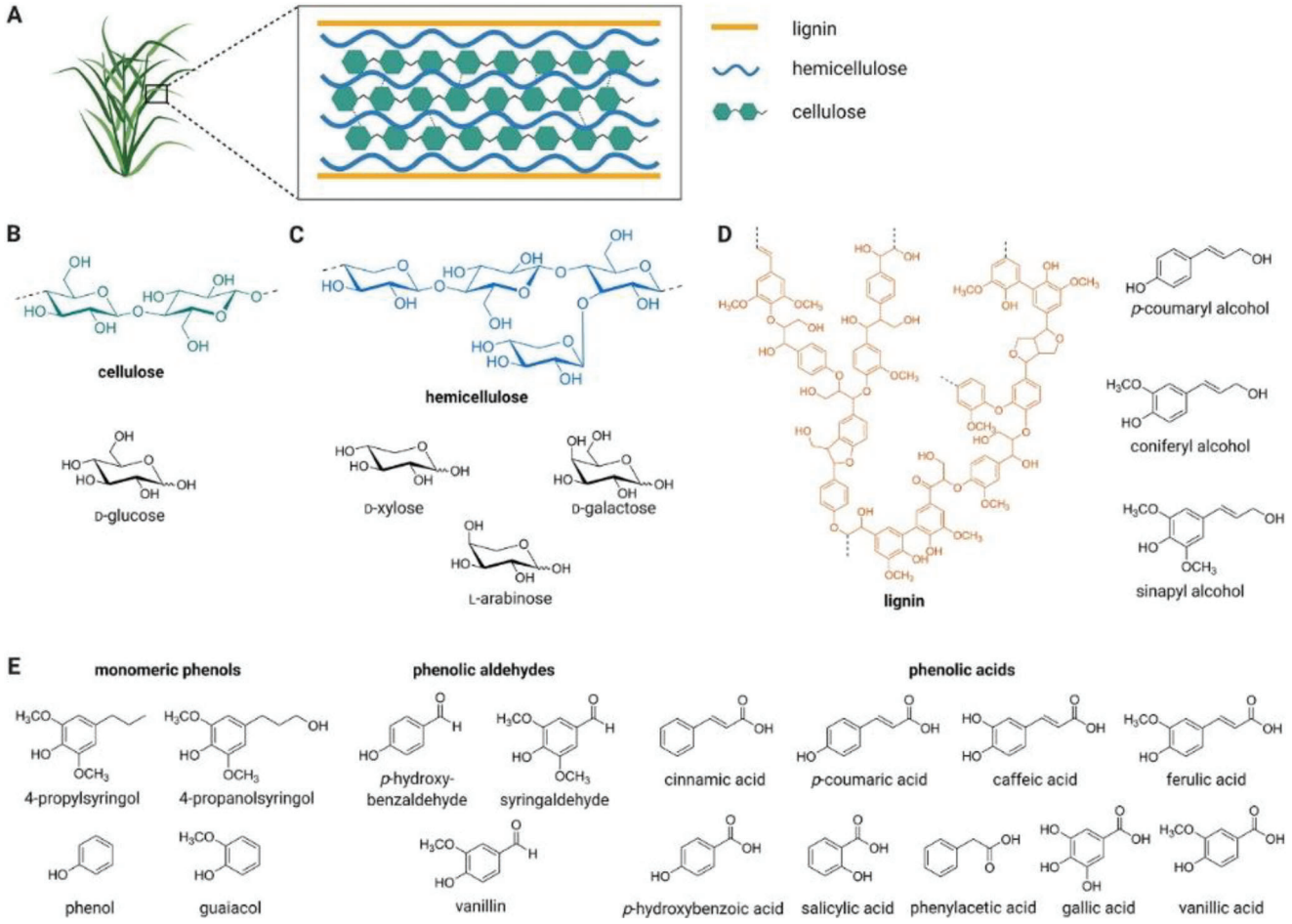

Figure 2. A) Recalcitrant and complex structure of the biomass. B) Cellulose biopolymer is mainly composed of glucose. C) Hemicellulose is predominantly composed of xylose, galactose, and arabinose sugars. D) Lignin biopolymer is made up of phenolic. E) Phenolics derivatives obtained from lignin after pretreatment to the biomass. Adapted with permission.[39] Copyright 2021, American Chemical Society.

production should be optimized in an integrated manner by considering upstream (pretreatment and the construction of strains), midstream (fermentation), and downstream (isolation and purification of PHAs polyesters from microbial biomass).

Herein, we reviewed the current status and limitations of PHAs production via the conventional biorefinery approach. Particular emphasis is put on the development of integrated lignocellulosic biorefinery, which can improve PHA's production costs, diversify product portfolio, and promote the circular economy by efficient conversion of biomass and zero waste emission. The general strategies of the co-production of microbial PHAs with value-added chemicals have been previously proposed[31,32] and were used as a guideline for the development of integrated biorefinery processes. The numerous co-production strategies for the simultaneous PHAs and value-added chemicals are presented to demonstrate how integrated biorefinery approaches are successfully developed for PHAs production in practice. To guide readers to better understand how integrated biorefinery approaches are helpful to achieve better environmental sustainability and economic feasibility in comparison to conventional approaches, a detailed study of the life cycle, and techno-economic analysis of integrated upstream and downstream processes are provided. Finally, the future challenges in further advancing integrated biorefinery and perspectives on successfully establishing this technology for real-world applications are discussed.

2. Bottlenecks of Conventional Biorefinery

The goal of the biorefinery is to produce green and sustainable products such PHAs biopolymer, biofuels, and platform chemicals from renewable lignocellulosic biomass that are promising substitutes for fossil-based products.[33] The sustainable and inexpensive carbon sources from lignocellulosic biomass can be obtained for the biosynthesis of PHAs via modification of cellulose, hemicellulose, and lignin, but their recalcitrant nature and complex structure limit their uses for large-scale PHAs production, as shown in **Figure 2**. To meet these needs and secure a sustainable future, cascade biorefinery processes used for the depolymerization of lignocelluloses to intermediates such as cellulose, hemicellulose, and lignin derivates that can be upgraded via biotechnological process to multiple bio-based products are under intensive research.[34] The fractionation of biomass into cellulose, hemicellulose, and lignin is the first step in the biorefinery. The current fractionation methods, including pretreatment used for biomass,

are less selective, generate inhibitors affecting the cell growth of microorganisms, low yield of sugars, and focus more on the maximum recovery of cellulose, but hemicellulose and lignin remain underutilized wastes in the biorefinery.[35,36]

The utilization of cellulose, hemicellulose, and lignin feedstock for the production of other value-added chemicals along with PHAs is very important for the implementation of the circular economy approach in PHAs production.[37] In this context, conventional pretreatments are not suitable to advance biorefineries for economic and environmental sustainability reasons. Additionally, the limited raw materials and technologies are the major disadvantages of the conventional biorefinery processes that restrict to use of a single feedstock, thus minimizing the opportunity to produce multiple products.[38]

To a large extent, the expensive pretreatment, generation of inhibitors in the hydrolysate, low-volume production of PHAs, inconsistent properties of the polymer, and lack of life cycle and techno-economic assessment data, these factors significantly hinder the industrial-scale production of PHAs from sustainable biomass.

3. Next-Generation Industrial Biotechnology (NGIB)

CIB approaches used for PHAs production from biomass are not economically viable because of high production costs associated with complicated fermentation processes such as sterilization, consumption of fresh water and energy, poor polymer properties, low volume of PHAs productivity, complex isolation and purification processes of PHAs polyesters.[40–42] Many research efforts have been devoted to overcoming such challenges and the most competitive approach is to develop "next-generation industrial biotechnology" based on rapid growth and contaminant-resistance extremophilic microorganisms, which allow open and continuous bioreactors and thus assist in economic PHAs production with superior polymer properties as compared to CIB approach.[6] *Halomonas* spp. is a well-known example of the production of PHAs under an open and continuous bioreactor proposed for NGIB.[40] Unlike CIB, the NGIB approach is AI-controlled, making it ideal for the production of superior quality polymer and maintaining a homogenous fermentation profile compared to inefficient batch processes in CIB.[41]

However, PHAs production costs are expected to be notably reduced by the NGIB approach, a further breakthrough should be addressed to realize a strong PHAs industrial value chain from upstream to downstream processes. To achieve this goal, extremophilic and robust strains need to be developed via advanced metabolic engineering and synthetic biology tools.

4. Sustainable Integrated Lignocellulosic Biorefinery Design

To improve the overall cost and environmental sustainability in PHAs production in comparison to the conventional biorefinery concept, the integrated biorefinery design has been proposed, which combined various biomass processing units as a whole.[35,43–45] Based on the above-mentioned definition, the sustainable integrated biorefinery design is a processing facility that efficiently converts biomass to various products such as biohydrogen, biosurfactants, proteins, amino acids, bioethanol, organic acids, and ectoines along with PHAs, as shown in **Figure 3**. PHAs co-production with these value-added products transforms biorefinery toward sustainability by efficient utilization of biomass and optimizing the overall capital costs of PHAs production.[31] The profitable and sustainable implementation of the integrated lignocellulosic biorefinery is depended on the following critical parameters.

1) Use greener technologies to ensure maximum biomass conversion efficiency and minimum waste generation so that we can claim that biorefinery-based PHA product is sustainable and green.
2) Able to utilize variable seasonal feedstock through an integrated biomass processing platform.
3) Maximum yield and superior quality of PHAs and co-products.
4) Investigate its economic feasibility and environmental sustainability via tech-economic analysis and life-cycle assessment tools.

4.1. Process Integration for the Production of PHAs

Industrial-scale production of lignocellulose-based PHAs production requires a facile and efficient biorefinery process that ensures environmental sustainability and economic viability.[23,37,43,45–48] The current paradigm for the biological valorization of lignocellulose to PHAs has involved four operational units: i) biomass pretreatment, ii) saccharification, iii) fermentation, and iv) downstream processing to recover and purify PHAs polyesters from microbial biomass.[37] These processes are segmented into different combinations, over time efforts have focused on the design of integrated strategies to reduce the overall process costs and improve sustainability in lignocellulose-based PHAs production.

Currently, most of the pilot-scale and industrial-scale processes are proceeding through separate saccharification and bioconversions.[49] The elimination of separations between hydrolysis and fermentation steps opens the door to the integration of these biomass processing units, through designing simultaneous saccharification and fermentation (SSF) and consolidating bioprocessing (CBP).[36] The pros and cons of SSF, CBP, and NGIB bioprocesses are shown in **Table 1**.

In the SSF approach, the commercial enzyme is used to carried-out saccharification of lignocellulose, subsequently, lignocellulosic hydrolysates can be converted into value-added products such as PHAs and bioethanol by using prokaryotic microorganisms capable of metabolizing fermentable sugars.[49] Nevertheless, the use of commercial enzymes can significantly increase the costs of PHAs production within a biorefinery.[50] On the other hand, the CBP approach combines pretreatment with saccharification and fermentation in a single step, thus reducing the costs invest in multiple processes.[49,51] Previous techno-economic assessments demonstrate that the implementation of CBP approaches in the biorefinery reduces 20% to 40% of costs as compared to conventional approaches.[56] Thus, the CBP approach is receiving much attention from the researcher as compared to the SSF approach. CBP approach includes either i) a

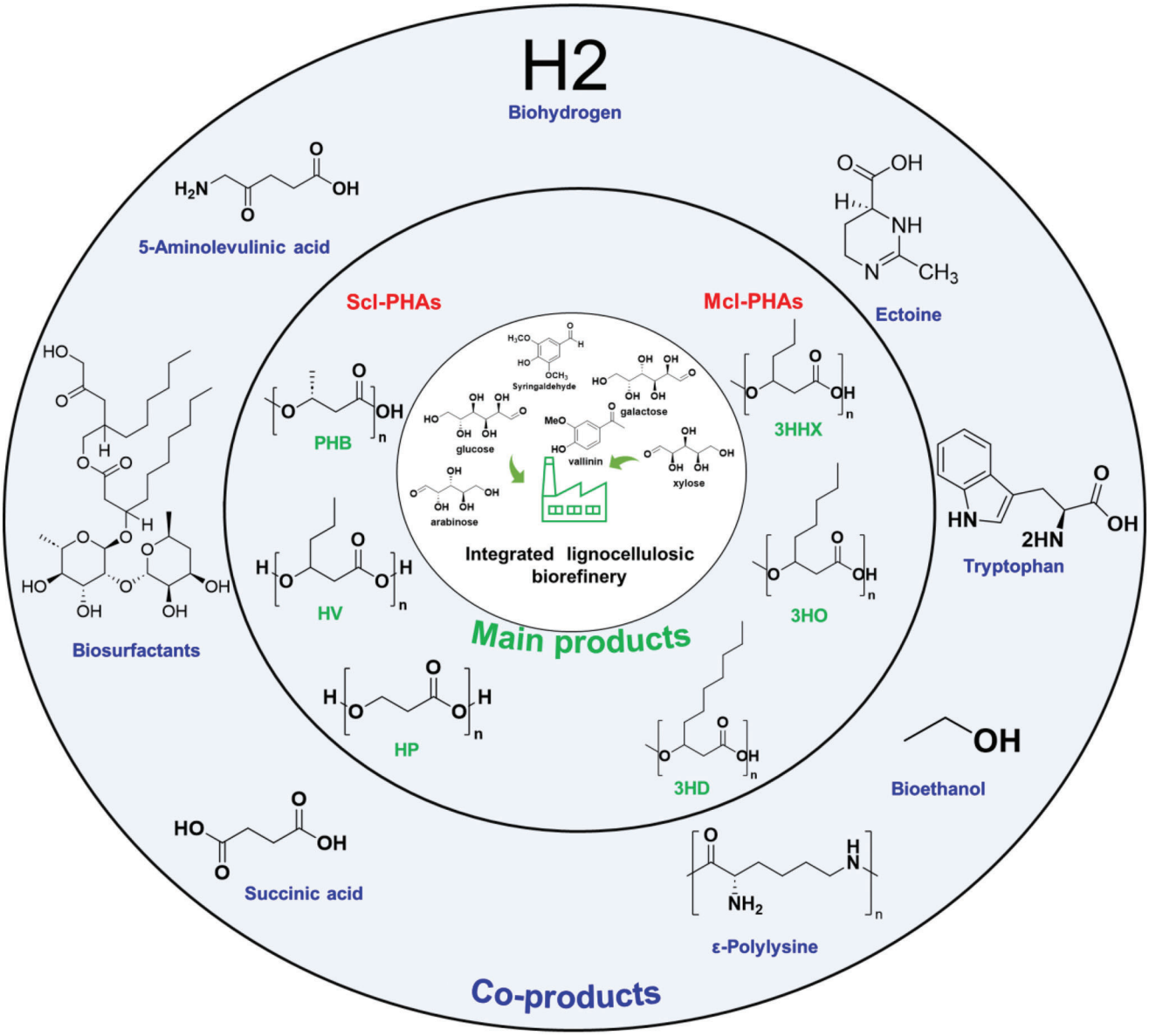

Figure 3. Overview of the multiple value-added co-products along with PHAs from bioconversion of fermentable sugars and phenolic compounds derived from lignocellulosic biomass via integrated lignocellulosic biorefinery approach, where Mcl-PHAs: medium-chain-length polyhydroxyalkanoates, Scl-PHAs: short-chain-length polyhydroxyalkanoates, PHB: poly(3-hydroxybutyrate), HV: poly(3-hydroxyvalerate), 3HP: poly(hydroxypropionate), 3HHX: poly(3-hydroxyhexanoate), 3HO: poly(3-hydroxyoctanoate), 3HD: poly(3-hydroxydecanoate).

genetically engineered strain to express and secret biomass hydrolyzing enzymes to convert it into fermentable sugars for the biosynthesis of desired products, ii) constructing desired products' metabolic pathways into the microbes that natively degrade biomass, or iii) integrating both approaches.[51,57] Salamanca-Cardona et al.[58] *Escherichia coli* JW0885 strain was engineered to overexpress xylanases and PHA-producing enzymes for the biosynthesis of poly(lactate-*co*-3-hydroxybutyrate) P[LA-*co*-3HB] from beechwood xylan as a consolidated bioprocessing strategy. Likewise, Salvachúa et al.[54] demonstrated the simultaneous depolymerization of lignin and catabolism of aromatic derivatives from lignin in bacteria to accumulate mcl-PHAs, as shown in **Figure 4**.

This study provides the foundation for the concept of lignin-consolidated bioprocessing, which may play an important role in the biological valorization of lignin. Furthermore, Govil et al.[18] reviewed strategies of CBP of biomass to PHAs production using thermophiles and highlighted that the use of thermophiles can assist to eliminate hazardous pretreatment and the addition of expensive enzymes for the hydrolysis, enabling efficient saccharification and fermentation of unprocessed biomass and direct to PHAs in a single step. Therefore, the development of efficient CBP technology for the conversion of renewable biomass to PHAs in a single step, eliminating expensive biomass pretreatment and enzyme hydrolysis step would be a great breakthrough for PHAs manufacturing industries.

Table 1. Comparison between SSF, CBP, and NGIB bioprocesses used for PHAs production with their pros and cons.

Bioprocesses	Advantages	Disadvantages	Ref.
SSF	• Operate at ambient temperature (30–50 °C), pH (4–5), and pressure • Hydrolysis of biomass and fermentation is performed in a single unit • Reducing time and capital costs of processes • Reduce the formation of inhibitory compounds from enzymatic hydrolysis and improve the performance of the process	• Incompatibility of optimum temperature and pH conditions for saccharification and fermentation step. • Less efficient in terms of productivity	[36, 52, 53]
CBP	• CBP is one pot that combines three major biomass conversion processes: enzymatic hydrolysis, fermentation, and PHAs production • CBP minimizes energy consumption, is cost-efficient, reduces waste generation, and is simplest process • Additional biocatalyst is not needed	• Scaling up CBP is challenging • The overall conversion rate and productivity are low • Implementation of the CBP approach is often required genetic modification in bacteria which is complicated and time-consuming	[18, 51, 54]
NGIB	• Simple and cost-effective process • Contamination-resistant strains • Reduce energy consumption • Replace fresh water with seawater	• Implementation of the NGIB approach often needs genetically engineered bacteria which is a complicated and time-consuming process • Regulatory challenges	[6, 28, 55]

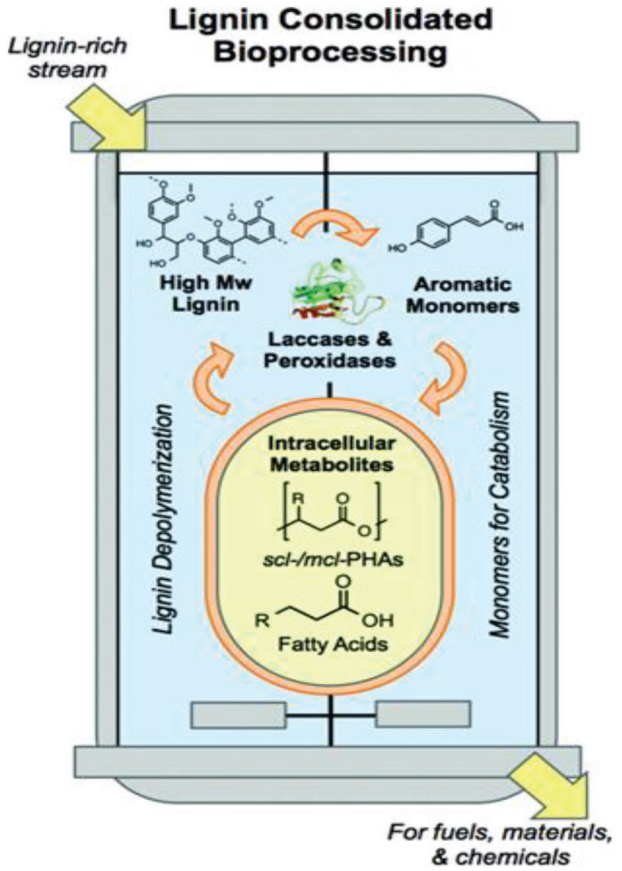

Figure 4. Demonstrate the concept of lignin-consolidated bioprocessing in which a lignin-rich waste stream is provided to microbes that secrete a ligninolytic enzyme to depolymerize high-molecular weight lignin into aromatic monomers and microbes uptake it to co-production of PHAs with value-added fine chemicals. Adapted with permission.[54] Copyright 2015, Royal Society of the Chemistry.

However, CBP approaches offer more opportunities for making the bioprocess of PHAs more economically viable and sustainable, the challenge remains the development of robust microbial biocatalysts capable of uptake fermentable sugars and lignin to produce desired products with a yield that are industrially relevant. Metabolic engineering and synthetic biology tools and strategies play an important role to solve this challenge.

4.2. Metabolic Engineering and Synthetic Tools to Design Integrated Biorefinery

Biorefinery processes are divided into four parts: the first, upstream process involves the depolymerization of biomass into renewable feedstocks, the second, upstream process involves the development of microbial strains capable of efficiently utilizing renewable and inexpensive carbon sources to produce the desired products, the midstream process involves the cultivation of microorganisms and produced desired products (including PHAs), the downstream process involves recovery and purification of the desired product. Among these four processes, the upstream process involves the development of microbial strains that are very important because it determines the overall efficiency of the conversion of biomass feedstock into products.[59] Generally, microorganisms for the fermentation processes are obtained from nature and they are not optimized to produce desired products. Herein, metabolic engineering and synthetic biology play a vital role.[28] Metabolic engineering and synthetic biology can be defined as the use of genetic engineering to modify the metabolism and redesign of microorganisms to achieve specified objectives.[37] These objectives include improving the yield of desired products (PHAs), and the co-production of bio-based fine chemicals along with PHAs that wild strains could not produce due to a lack of metabolic capability to synthesize multiple products. Metabolic engineering and synthetic biology can be performed to allow the microorganisms to utilize seasonal variable biomass feedstocks. Furthermore, these tools can be applied to reduce the production of by-products, thus it makes the downstream process easier and improving the yield of the desired product (PHAs) with tailored properties, as shown in **Figure 5**.[28,30] With the most recent advancement in metabolic engineering, it becomes possible to integrate synthetic biology, system biology, and evolutionary engineering that promote the development of superior microbial strains capable of co-production of microbial PHAs with value-added chemicals. Cho

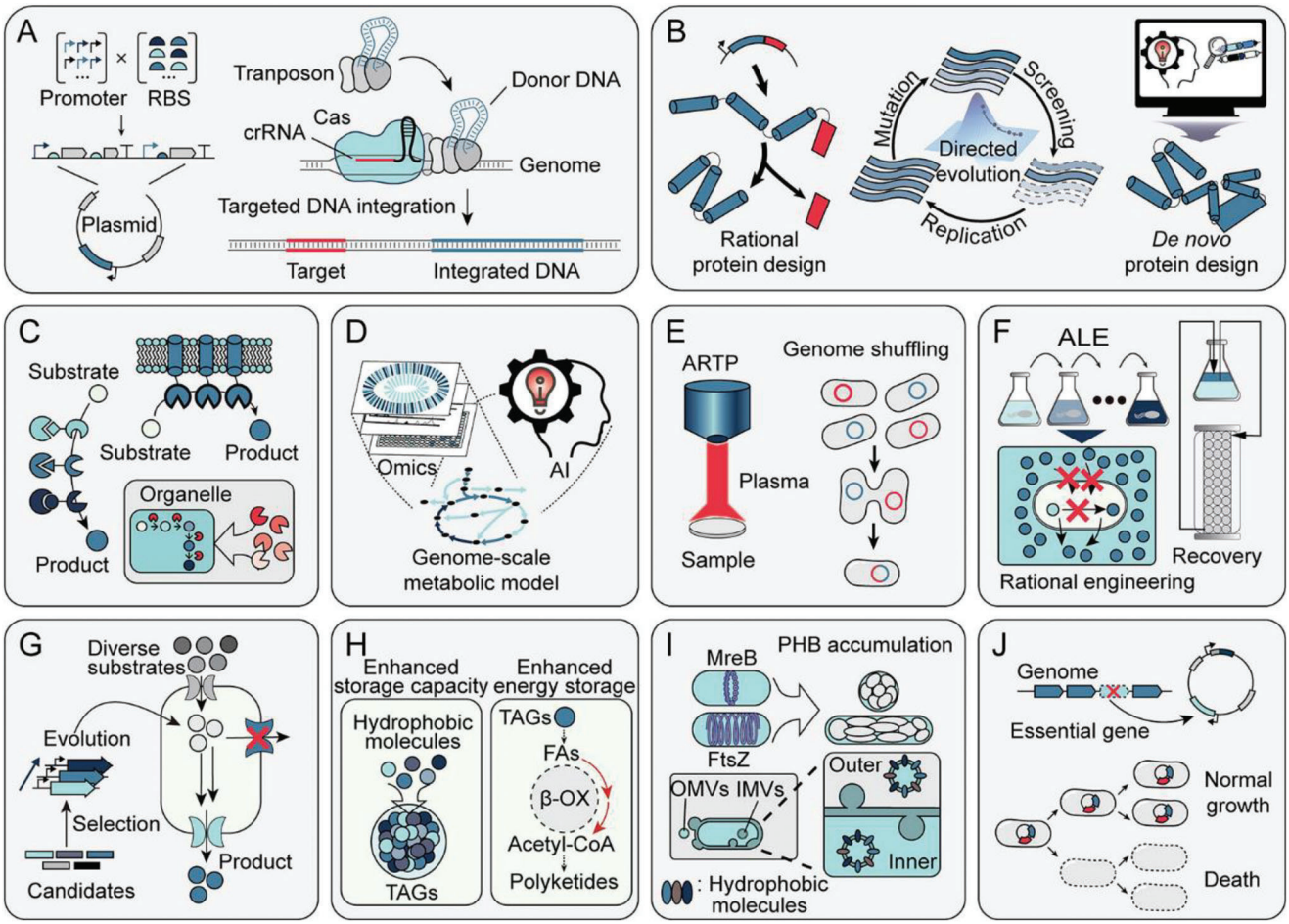

Figure 5. Illustrated advanced metabolic engineering and synthetic biology tools and strategies for the production PHAs and co-products such as biosurfactants, organic acids, proteins, amino acids, biohydrogen, and ectoine along with PHAs production: A) Molecular tools used for the construction of metabolic pathways and introduce into the host cells. B) Enzymes engineering using rational design, de-nova pathways construction. C) Substrate controlling strategies to product formation. D) Genome-scale metabolic model determined by omic and artificial intelligent data. E) Chassis random mutagenesis approach via room-temperature plasma and genome shuffling technique. F) Enhance tolerance to the desired product via adaptive laboratory evolution (ALE) and rational engineering. G) Transport engineering strategies to change metabolites distributions. H) Enhance storage capacities for metabolites and energy. I) Increases cell size of microbes via morphology engineering to accumulate more PHAs. J) Antibiotic free selection. Adapted with permission.[1] Copyright 2022, American Chemical Society.

et al.[1] and Ko et al.[59] reviewed different metabolic and synthetic biology strategies to develop microbial cell factories, equivalent to petroleum refineries, capable of efficiently producing multiple sustainable products using renewable biomass. Also, discussed are the challenges in improving metabolic engineering and synthetic biology and perspectives on successfully establishing this technology in biorefineries. For a more detailed study of metabolic engineering and synthetic biology, the readers are advised to refer to the reviews of Cho et al.[1]and Ko et al.[59]

4.3. Coproduction Strategies of PHAs with Value-Added Chemicals

With the most recent advancement in metabolic engineering and synthetic biology, it is possible to construct high-performance strains capable of producing multiple products from renewable biomass. As illustrated in **Figure 6**, the coproduction of PHAs with value-added chemical significantly reduces the costs of raw materials, make its downstream process easier, and improve the yield of the desired products (PHAs).[38,39] The coproduction strategies and biosynthetic pathways of coproduction of PHAs with biohydrogen, amino acids, proteins, biosurfactants, and other final chemicals are depicted in Figure 6. Notably, if the co-production of PHAs with value-added fine chemicals can be obtained in high yield without affecting the metabolism of cellular balance, this approach improves the process costs associated with fermentation.[60]

4.3.1. Coproduction of PHAs with Biohydrogen

Recently, biohydrogen has been considered a key compound for the production of renewable energy.[61] Biological H_2 production offers clean and green processes for energy production. The biological production of H_2 via dark fermentation

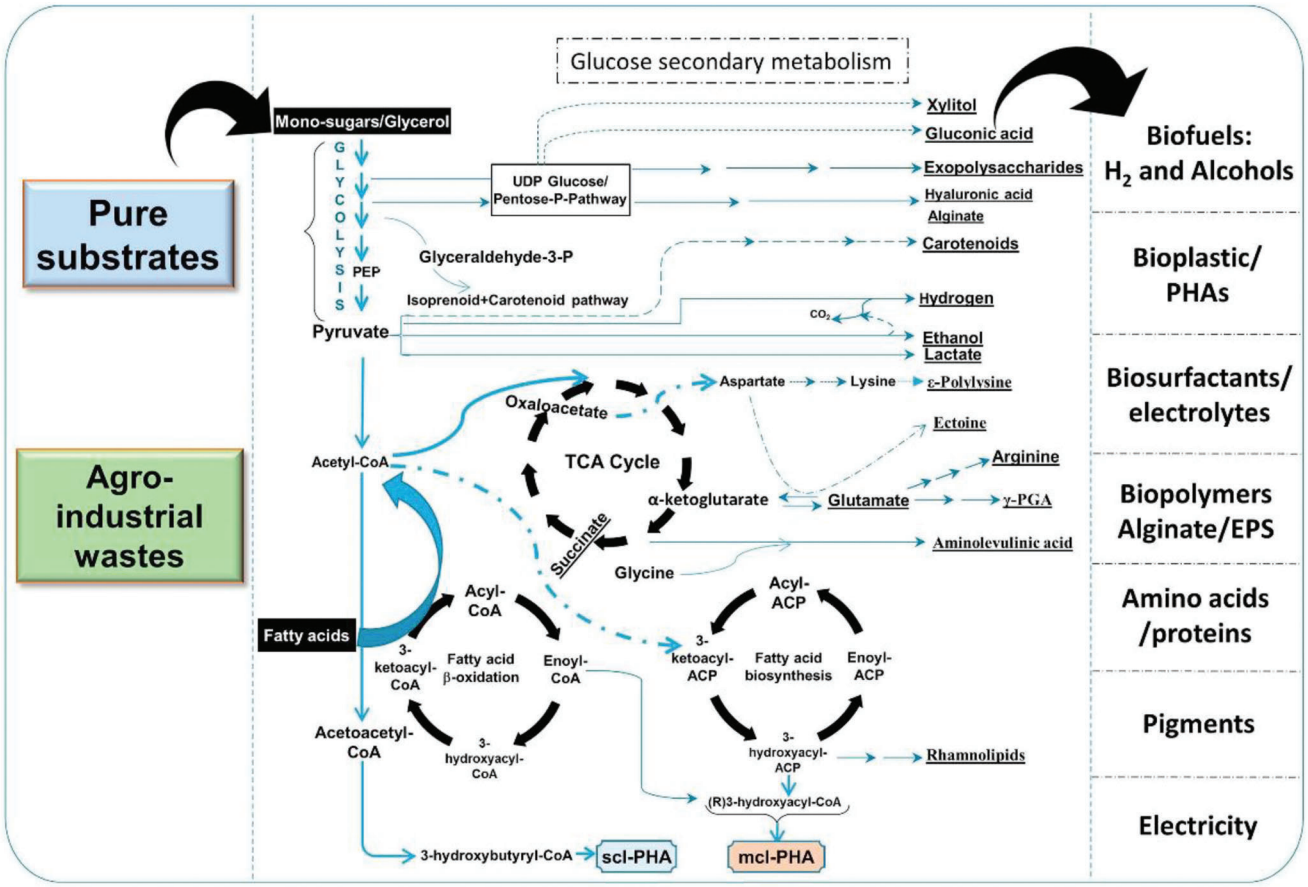

Figure 6. Schematic representation of biosynthetic pathways of co-production of PHAs with biofuels, biosurfactants, amino acids, proteins, pigments, and bioelectricity. Reproduced with permission.[32] Copyright 2018, Elsevier.

has received more attention due to, less energy consumption and high H_2 evolution rates as compared to other routes of H_2 production.[31,61–63] Several prokaryotic microorganisms such as *Rhodobacter sphaeroides*,[64] *Bacillus* sp,[64] recombinant *Escherichia coli*,[64] and *Rhodopseudomonas palustris*[65] have been reported with the ability to coproduction of scl-PHAs and biohydrogen gas, as shown in Figure 6. Genetically modified bacteria such as *Escherichia coli* were reported for their ability to coproduction biohydrogen and PHAs under anaerobic conditions; these bacteria metabolized glucose and transferred glucose to phosphoenolpyruvate, pyruvate, formate, and acetyl-CoA. Pyruvate was catalyzed either to lactate via lactate dehydrogenase, to form acetate via pyruvate oxidase (*ldhA*), or to formate and acetyl-CoA via pyruvate oxidase (*pox*). Herein, formate can act as an important precursor for biohydrogen production, which is converted into biohydrogen by the formate hydrogen lyase system.[31] Acetate can be converted to acetyl-CoA via the enzyme acetyl-CoA synthetase (*aACS*), which can act as a precursor for PHAs production. Acetyl-CoA molecules condensed to form PHAs. This biological reaction is triggered by three enzymes, namely, PHA synthase (*phaC*), beta-keto thiolase (*phaB*), and acetoacetyl-CoA reductase (*phaA*). To minimize by-product formation and enhanced biohydrogen production, some genes in bacteria were deleted and genes such as *hyd* 3 and *acs*A were overexpressed, thus improving the simultaneous production of biohydrogen and PHAs.[66] The integrative approach for the simultaneous production of PHAs and biohydrogen is highly beneficial since hydrogen production accelerates the conversion rate of pyruvate to acetyl-CoA thus, increasing scl-PHA accumulation. Similarly, the acetyl-CoA utilization by PHAs synthesis boosts pyruvate degradation which intensifies carbon flux to formate formation and subsequently to hydrogen production. The overexpressed hydrogenase 3 also intensifies metabolic flux from formate to hydrogen production, thus improving hydrogen production.[31]

4.3.2. Coproduction of PHAs with Biosurfactant

Biosurfactants are long-chain aliphatic or hydroxy fatty acids that are synthesized by microorganisms using renewable feedstocks. Based on their chemical structure and origin, biosurfactants are classified into glycolipids, phospholipids, lipopeptides, and polymeric surfactants.[67] Among them, glycolipids and phospholipids show increasing attention as emerging bio-based surfactants because they are considered green alternatives to chemical surfactants, and have the potential to replace chemical surfactants in medical, health, agriculture, and cosmetic applications.[68] Its high production costs in comparison to chemical surfactants are the major limitation to their broader industrial applications.[69] To overcome such a challenge, the

integrative approach for the simultaneous production of PHAs and biosurfactants has been developed, as shown in Figure 6. Microorganisms such as *Enterobacter aerogenes*,[70] *Halomonas* sp.,[71] and *Pseudomonas* sp.[72,73] Arumugam et al.[70] utilized *Calophyllum inophyllum* oilcake as an inexpensive and renewable carbon source for the simultaneous production of PHAs and rhamnolipid by using anaerobic *Enterobacter aerogenes*. The authors reported yields of PHAs 4.2 g L^{-1} and rhamnolipid 5.8 g L^{-1} under optimum conditions, i.e., inoculum size 5% v/v, pH-6.5, and C:N (5:1). This integrative fermentation strategy reduced costs of raw materials and downstream processing.

PHAs and biosurfactants were biosynthesized through two different metabolic pathways: i) fatty acids de nova pathways which generate 3-hydroxyethyl-CoA, and ii) fatty acid degradation via β-oxidation is the key metabolic pathway of fatty acids.[74] Both metabolic pathways produce (R) 3-hydroxy acyl-ACP precursor for the synthesis of PHAs and rhamnolipid. 3-hydroxyaceyl-ACP precursor converts into β-hydroxyalkanoyl-β-hydroxyalkanoic via the bio-chemical reaction catalyzed by HHAs (*rhlA*) synthetase. Then, β-hydroxyalkanoyl-β-hydroxyalkanoic was converted into mono-rhamnolipid and di-rhamnolipid via rhamnosyl transferase I (*rhlB*) and rhamnosyl transferase II (*rhlC*).[31]

4.3.3. Coproduction of PHAs with Amino Acids and Proteins

The cost of PHAs could be reduced by recovering other products released during PHAs production. Among them, proteins in high amounts are released in the liquid stream after isolating PHAs polyester from microbial biomass. The protein accounted for 50% of the cell dry mass of the bacteria, and this percentage varied and largely depended on the PHAs contents accumulated in the cells and microbial biomass.[75] Once soluble proteins and PHAs are released from microbial biomass, they can be easily separated via centrifugation, sedimentation, or filtration.[76] The recovered protein has many applications as animal feed, adhesive, etc.[77] The reuse and recycling of the protein maintained the circular economy approach in PHAs production.[78]

Besides protein recovery, intensive efforts have been focused on the recovery of individual amino acids along with PHAs production.[30,79,80] Amino acids production along with PHAs receives increasing attention because it has many applications in medicine, food, and animal feed.[81] Gu et al.[80] improved the production of L-tryptophan by introducing *phaCAB* operon genes from *Ralstonia eutropha* into L-tryptophan-producing *E. coli* GPT1002. The expression of the PHAs synthesis gene caused PHAs accumulation inside cells and enhanced L-tryptophan production. The addition of xylose as a co-substrate increased precursor supply for PHAs synthesis and enhanced L-tryptophan production. The authors reported a yield of L-tryptophan of 14.4 g L^{-1} and 9.7% w/w PHB. Notably, PHAs synthesis might alter the cellular metabolisms by changing cellular oxidation/reduction potential and metabolic flux in the microorganism.[82] In addition, it was observed that the expression of the PHAs synthetic pathway improved L-isoleucine production. When *phaCAB* for PHAs synthesis overexpressed in L-isoleucine-producing *Corynebacterium glutamicum* WM001, recombinant strain lowered metabolic flux toward other by-products.[82] As a result, reserved and blocked carbon flux could be moving toward L-isoleucine production through the tricarboxylic acid (TCA) cycle. It was found that the recombinant strain increased the 65% yield of L-isoleucine with the yield of PHBV 15 g L^{-1} in comparison to the wild strain. Remarkably, the co-productions of PHBV have a positive impact on the transcription of a key enzyme, enhancing the yield of the product, altering the cofactor flux, and improving cell growth.

5-Aminolevulinic acid (ALA) is a key cell metabolic precursor that has applications in cancer treatment, biodegradable pesticides, plant growth regulators, and important intermediate for the biosynthesis of cytochrome, hem, and vitamin B12.[83–85] The chemical synthesis of ALA is more complex and has a low yield.[84] Therefore, the biosynthesis of ALA has received more attention. Li et al.[86] cloned mitochondrial ALA synthase *gene hem1* and ALA synthase from *Saccharomyces cerevisiae* and expressed in *Escherichia coli* (*E. coli*) through the plasmid encoding T7 expression system with a T7 RNA polymerase. Engineered *E. coli* expressing biosynthetic pathways of PHB and ALA have led to 43% of the accumulation of PHB inside the cells of *E. coli* and 1.6 g L^{-1} of extracellular production of ALA. Furthermore, the authors grew recombinant *Halomonas* TD in unsterile and continuous conditions to produce PHB and ALA simultaneously, resulting in 22% of PHB produced intracellularly and 635 mg L^{-1} of ALA produced extracellularly. This work has demonstrated an effective strategy for improving the simultaneous production of PHAs and ALA. The use of an NGIB such as unsterile and continuous fermentation conditions could help to reduce PHA's production cost. In another study, Zhang et al.[87] used single-cell biorefinery as an attractive strategy for the simultaneous production of PHAs and ALA. In this study, the authors integrated multiple copies of the PHAs synthesis operon into the ALA-producing *E.coli* $\alpha\Delta$poxB strain. This recombinant *E.coli* harbors the genes *hemA and hemL* responsible for ALA synthesis via the C5 pathway, resulting in the co-production of PHB (accumulated 38.2% of cell dry mass) and extracellular ALA (3.2 g L^{-1}). Furthermore, the same authors have integrated poly(3-hydroxybutyrate-*co*-hydroxylate) (PHBV) biosynthetic pathways into *E. coli* and co-expressed with *HEMA and hemL*; the strain accumulated 39% of PHBV with 10.3% mole HV fraction along with 3.0 g L^{-1} of ALA extracellularly produced. The simultaneous production of PHB, PHBV, and ALA boosts the biorefinery toward sustainability by improving carbon efficiency and costs of PHAs production.

ε-Polylysine is an interesting homopolyamide because it is biodegradable and nontoxic to humans, thus showing potential application as a drug carrier, gene carrier, hydrogel, and coating material.[88] Bhattacharya et al.[89] synthesized PHBV and ε-polylysine simultaneously by *Bacillus licheniformis* PL26 using crude glycerol as an inexpensive and renewable feedstock. The authors reported bacteria accumulated 65% PHBV of its cell dry mass and yielded 0.2 g L^{-1} of ε-polylysine.

4.3.4. Coproduction of PHAs with Bioethanol

Coproduction of PHAs with bioethanol was shown by Silva et al.[90] According to the authors, the integration of bioethanol into lignocellulosic biorefinery would provide a huge amount of xylose, which can be used as an inexpensive and renewable source for the production of PHAs. The co-production of PHAs in a bioethanol plant may reduce the production costs of

bioethanol. Syngas fermentation and microbial PHAs production processes have been developed over the decades but never integrated these two processes. Notably, Lagoa-Costa et al.[91] carried out anaerobic bioconversion of syngas by *Clostridium autoethanogenum* to produce bioethanol and acetic acid. Subsequently, acetic acid from an anaerobic syngas fermentation was converted into PHAs by using mixed microbial consortia. Furthermore, Liu et al.[35] designed a plug-in process for the bioconversion of lignin to PHAs, where carbohydrate fraction left after lignin solubilization was used for ethanol production. The author's work showed that the co-production of PHAs in cellulosic ethanol plant reduce the PHA's selling price to as low as \$6 kg^{-1}.

4.3.5. Coproduction of PHAs with Carboxylic Acids

Levulinic acid is one of the important biomass-derived platform chemicals.[92] Coproduction of levulinic acid takes place through pentose sugars could be converted to pyruvic acid through the pentose phosphate pathway (PP pathway).[43] Subsequently, the transformation of pyruvic acid to levulinic acid has been reported.[93] The pyruvic acid is transformed into levulinic acid through two pathways: i) conversion of pyruvic acid to 4-oxo-2 hydroxy-pentatonic acid, which dehydrated into 4-oxo-2 pentatonic acid that further reduced into levulinic acid, ii) pyruvic acid converted into acetyl-CoA via oxidation, it undergoes Krebs cycle to produce levulinic acid from succinyl CoA.[93] Pinto-Ibieta et al.[94] demonstrated the simultaneous production of levulinic acid and PHA by a mixed microbial community feeding hemicellulose hydrolysate as an inexpensive carbon source. The authors reported the yield of levulinic acid was 37% w/w and 22% of PHB was accumulated when hemicellulose hydrolysate was enriched with 50% of acetate.

Succinic acid is a dicarboxylic acid.[95] It is considered an important building block for the production of bulk chemicals and wide applications in pharmaceuticals, cosmetics, and food industries as flavoring agents. There are some companies such as BASF operating bio-succinic acid plants at a commercial scale. However, its selling prices are higher compared to petroleum-based succinic acid (2 to 3 \$ kg^{-1}), and it is expected to increase by 19% in the coming years.[95] The coproduction of succinic acid with PHAs reduced its production costs. Kang et al.[96] designed and constructed pathways for the simultaneous production of PHB and succinate by genetic modification of the host. The authors developed succinate production pathways in *E. coli* with the aim of accumulation of acetyl-CoA which is the key precursor for PHA production. *E. coli* acetyl-CoA is produced from pyruvate and enters the TCA cycle for energy and cells intermediate production.[97] Under the anaerobic condition, succinate is formed by succinyl-CoA synthetase, acting as an intermediate of the TCA cycle and further converted to fumarate via succinate dehydrogenase. To achieve succinate accumulation, it is important to inactivate the *sdha* gene (succinate dehydrogenase) to prevent the transformation of succinate to fumarate via the TCA cycle. Subsequently, *E. coli* metabolized large quantities of acetyl-CoA to accumulate PHB inside their cells. It was found that the inactivation of phosphotransacetylase/acetate kinase (*Pt*) and pyruvate oxidase (*PoxB*) prevents acetate secretion and allows more and more conversion of acetyl-CoA to PHB and succinate production. Additionally, a *ptsG* knockout was designed for several advantages: i) improve the phosphoenolpyruvate pool, ii) regulate the carbon metabolisms, and iii) prevent acetate production. The inactivation of the *icIR* gene, which encodes the glyoxylate operon *aceBAK* repressor, is used to activate the glyoxylate bypass. It results in more accumulation of succinate and less acetate formation. The metabolic pathway of synthesis of PHB was constructed in the host by subcloning the *phbCAB* operon from *Rlastonia eutropha*. The resulting recombinant *E. coli* strain efficiently utilized substrates with minimum by-product accumulation. On the other hand, PHB accumulation degraded a large amount of nicotinamide adenine dinucleotide phosphate (NADPH), speed up the TCA cycle, and was helpful for simulants succinate production. Using a novel strategy, the above authors could produce 24.6 g L^{-1} of succinate and 4.95 g L^{-1} of PHB simultaneously.

In another study, Amulya and Mohan[98] reported the integrated approach for the production of succinic acid and PHB using *Citrobacter amalonaticus*. The organic acids produced in the effluent after acidogenic fermentation are used to produce PHB by MMC. The authors reported the yield of succinic acid 14.7 g L^{-1} and PHB 24 g g^{-1}.

4.3.6. Coproduction of PHAs with Ectoines

Ectoines are one of the important biomaterials which are compatible solutes important for extremophiles to survive in extreme environmental conditions[99] and also have wide applications in the cosmetic, medical, and food industries.[100] Increasing demand for ectoines in the commercial market encourages researchers to develop sustainable and cost-effective routes for the large-scale production of ectoines from microbial resources. The integration of ectoines with PHAs production is beneficial because this approach shows a positive impact on the cost of both products. Several halophilic bacteria have been reported for the simultaneous production of PHAs and ectoines. Recently, Ma et al.[101] used next-generation industrial biotechnology platform for the simultaneous production of ectoines and PHB using recombinant *Halomonas bluephagenesis*, which grows at open unsterile and continuous conditions. The authors constructed de nova fatty acid pathways for ectoine into the chromosome of *Halomonas bluephagenesis* using two inducible systems which help to fine-tune transcriptional levels of three clusters relevant to the synthesis of ectoine, comprising *ectABC*, *lysC*, and *asd* according to a green fluorescent protein (GFP)-mediated transcriptional tuning approach. Integrated with bypasses deletion, the resulting recombinant *Halomonas bluephagenesis* achieved simultaneous production of ectoines 8 g L^{-1} and PHB 32 g L^{-1}, respectively. The co-production of PHB with small ectoines improves the process costs.

5. Techno-Economic and Sensitivity Analysis

Techno-economic analysis (TEA) is a method of assessment that allows researchers and industries to evaluate the costs and profits of a certain biorefinery or plant run with specific process kinetics. In this regard, cost analysis is done to gauge the costs associated with raw materials, equipment, utility, product selling

price, profit analysis, and the economy of the process.[78] The mentioned outcomes will provide insights into the underlying factors influencing PHA market price, which is crucial to its commercialization. In simpler terms, TEA gauges the economic feasibility of PHA production from lignocellulosic wastes. Sensitivity analysis is an assessment to understand the major factors that influence the overall economic model and make tweaks to optimize the process for better future economic purposes.[102]

Detailed research by Manikandan et al.[103] has reported that the estimated PHA cost price is 24 USD kg^{-1}. Their study used carob pods and pure sugars as feedstock in different setups. Their setup's capital cost was ≈417.15 million USD, while the annual operating cost was 1437 million USD. Their setup comprises stirred tank bioreactor (STBR) and an annual bioreactor (ABR), 50 units each. It was estimated that the setups could generate PHA per annum in tonnes, 18895.5 (STBR setup) and 30267.0 (ABR setup). The estimated net revenue was 312.37–509.37 million USD per year, and the pay-out period was 4.8–12.6 years, depending on the setup.

Pavan et al.[104] investigated the TEA of PHA production from citric molasses using different routes for product recovery. Their extraction methods studied were ultrasound, high temperature, high pressure, and no pretreatment as a control. Through their investigation, the capital cost for producing 2000 tonnes of PHAs annually was 33.12–36.02 million USD, with the high-temperature scenario being the lowest value obtained for total capital investment. The annual operating cost falls between 8.55 and 9.45 million USD per year. The pay-out period was 12.9–23.9 years, with the high-pressure scenario being the shortest. The PHA cost for the high-pressure scenario was 4.28 USD kg^{-1}. Besides using SA, Pavan et al.[104] also reported that up-scaling the production capacity to 10 000 tonnes per year resulted in a decline in production cost and pay-back period while increasing the internal rate of return. The cost of PHA was 2.73 USD kg^{-1} at this point.

Another study focused on the TEA of food waste valorization for integrated PHAs and biofuel production.[105] The capital investment was estimated at 42.83 million USD, with 8.55 million USD and 13.88 million USD per year for total equipment and annual operating costs, respectively. Using STBR, the estimated PHA generated per annum is 662.3 tonnes, with an estimated revenue of 3.64 million USD per year. The pay-out period is 7.31 years. The minimum selling price of PHA in this study was 4.83 USD kg^{-1}. It was mentioned that the fluctuation in the price of certain variables affected the minimum selling price of PHA. Based on the sensitivity analysis, the solid loading price and 2,3-butanediol price significantly impact the economic factors. 2,3-butanediol is a major byproduct found in this study. Thus, the selling price of 2,3-butanediol plays a significant role in the economic feasibility analysis. To this study, the higher the solid loading and price of PHAs and 2,3-butanediol, the better the economic values. Hence, positive net present value and vice versa.

Conclusively, the economic evaluation of the PHA's production process, up-scaling of production capacity, extraction processes and efficiency, and market conditions are some crucial elements that can directly impact the economic feasibility of PHAs. Reducing the production cost and capital investment can ultimately make PHAs an attractive asset from an economic point of view.

6. Life Cycle Assessments

With desirable properties such as biodegradability and biocompatibility, PHA has been introduced as an alternative to conventional oil-based plastics such as polyethylene terephthalate, polypropylene, and polyethylene.[106] This has resulted in a large driving force for the in-depth study of the production of PHA from agro-industrial residues via various techniques (e.g., pure microbial culture, mixed microbial culture, thermophilic PHA production),[107,108] as well as the environmental and economic assessment of these techniques.

Life-cycle assessment (LCA) is a technique used to identify and quantify the potential environmental impacts associated with a process, product, or service throughout its life cycle.[109] LCA is performed according to the iterative procedure described in the International Organization for Standardization (ISO) ISO 14040 and ISO 14044, starting with i) goal and scope definition (specifying system boundaries, impact category, and functional unit), ii) life cycle inventory (mapping of the flow of system), iii) life cycle impact assessment (translation of inventory data into different impacts categories), and iv) interpretation of data obtained.[110]

Previous reports showed that the overall PHA production life cycle required high energy in upstream (feedstock cultivation) and downstream processes (PHA production).[45,110] From 2000 to 2010, the research focused on evaluating the feasibility of PHA production and comparing it with oil-based plastic. In the present decades, scientists have driven the research focus to compare PHA production from different feedstocks and process parameters. Therefore, various LCA studies were performed to study the environmental impacts of these methods.[111]

Conventionally, PHA is produced via microbial fermentation in pure or open microbial cultures. The common substrates used are refined sugars, noble oils, or fatty acids, which were reported to account for nearly 50% of the total production cost of PHAs.[112] Therefore, scientists have made efforts to seek inexpensive substrates.[113] A review conducted by Sen and Baidurah in 2021[114] summarized the various types of carbon sources utilized for PHA production and noticed that inexpensive feedstocks (e.g., lignocellulosic biomass, algal, and bran based) produced lower PHA yield (26.7–55.6%) compared to the purified substrate (e.g., fat, oil, and glycerol based) (52.4–81.3%). However, environmental impacts were not studied. Hence, several efforts have been made to optimize the upstream processing of feedstocks and their ecological effects.

Among them, microbial fermentation by mixed culture for PHA production has been introduced. The advantage of this method over pure or open culture is the ability to utilize complex and inexpensive feedstock as substrate without the need for a sterilization process as in pure cultures, such as industrial wastewater, pyrolytic products of wastewater treatment sludge, and food waste[115–117] conducted an LCA comparison of PHA production from pure (corn) and mixed (industrial wastewater) cultures via microbial fermentation. However, the best method was not indicated, but the authors provided several recommendations to improve the production of PHA by mixed culture, including the selection of system boundaries (cradle-to-gate LCA is sufficient for PHA production with the same function at the gate; otherwise, cradle-to-grave LCA is suggested).

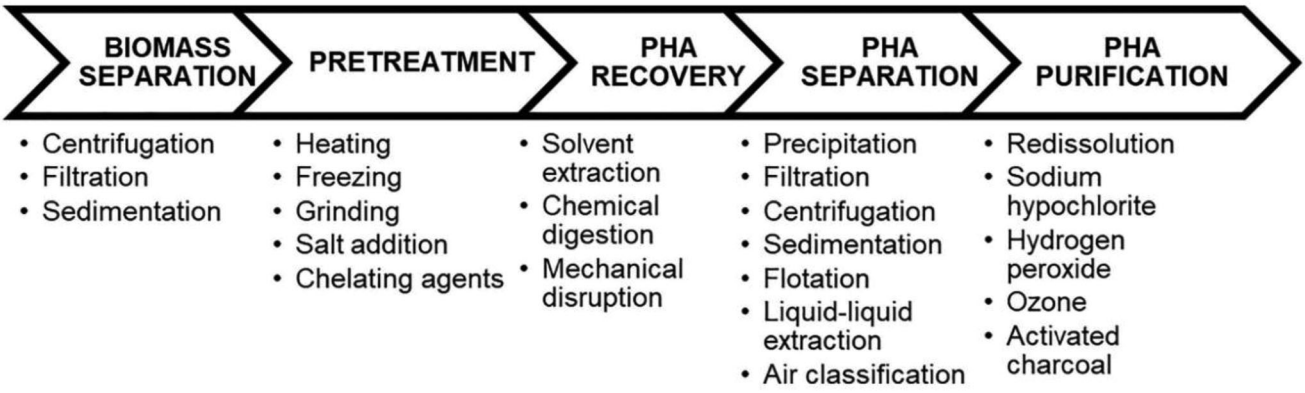

Figure 7. The process flow of PHA downstream processing and the standard methods for each step were extracted. Reproduced with permission.[111] Copyright 2021, Elsevier.

Recently, hybrid methods have been introduced in the upstream processes. Among them, mixotrophic cultivation of microalgae or cyanobacteria has been proven to improve the yield of PHA by two- to ninefold;[118] a photoautotrophic–heterotrophic consortium of cyanobacteria/microalgae and bacteria helps to reduce the wastewater treatment (organic waste from wastewater) efforts naturally;[119] and two-module system (1st module: photoautotrophic cyanobacterial growth to produce substrate; 2nd module: heterotrophic bacteria utilizing substrate made from 1st module for PHA production) can compensate for the low PHA yield production from fast-growing photoautotrophic cultivation.[120] Although these proposed methods demonstrated promising results for PHA production, the evaluation of these methods in the context of environmental aspects was not well-developed. Thus, LCA should be performed to identify the feasibility of these promising methods for PHA production and reduce the cost of the upstream processes.

Downstream processes of PHA comprise several steps, as shown in **Figure 7**. The recovery and purification of PHA produced are known to account for about 50% of the overall PHA manufacturing cost.[121] Few studies have been conducted on the environmental impact of MMC-based PHA production.

The research was carried out by Cora Fernández-Dacosta et al.[122] comparing three downstream recovery processes of PHA from wastewater (e.g., surfactant-hypochlorite, alkali treatment, and solvent-based extraction). The research reported that alkali treatment was most favorable from both economic and environmental points of view and does not require additional steps to recover the solvent used. Saavedra de Oso et al.[111] screened the peer-review articles and patents to evaluate and optimize the environmental performance of PHAs in downstream processing. The research reported that chemical digestion had advantages over solvent extraction, including lower production cost (up to 47% reduction) and avoidance of a highly energy-intensive solvent recovery step. Mechanical disruption (H2) was reported to contribute minimally to the environmental impacts, as shown in Figure 6, as compared to chemical digestion (H3) and solvent extraction (H1 & H4). H2 was also reported to be the most promising method for high-grade PHAs production as it poses the lowest global warming potential (GWP) and human toxicity (0.806 kg CO_2 eq, 0.036 kg 1,4-DB eq) as compared to chemical digestion (2.394 kg CO_2 eq, 0.068 kg 1,4-DB eq) and solvent extraction (12.956 kg CO_2 eq, 0.331 kg 1,4-DB eq) per kg of PHA produced, and a minimal amount of surfactants and chemicals was used in H2 extraction processes. H1 and H4 are recommended for the production of extremely high-grade PHAs but may contribute to the production of chemicals and heat that bring negative impacts to the environment, with freshwater eutrophication being the most heavily impacted category due to ethanol production. Besides that, H1 is preferable over H4 as it shows lower environmental impacts due to the lower heat duty (43% lower), cooling duty (49% lower), and electricity (24% lower) in H1, as shown in **Figure 8**.

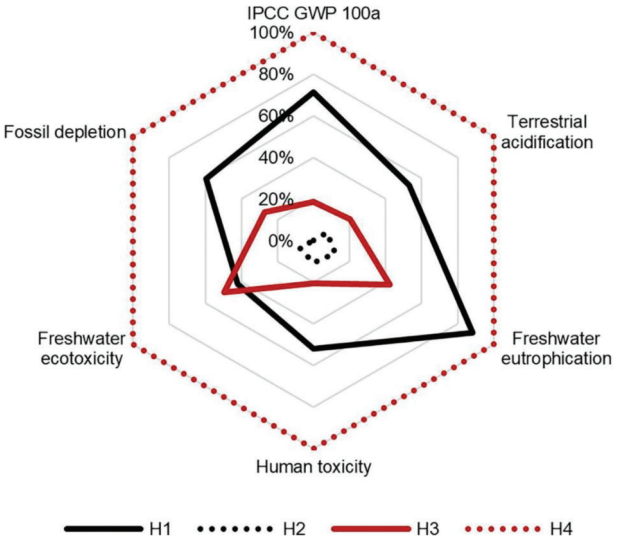

Figure 8. Comparative impact categories of different PHA extraction methods, where H1 and H4 represent chemical digestion, H2 represent mechanical disruption, and H3 represents solvent extraction. Reproduced with permission.[111] Copyright 2021, Elsevier.

However, most of the LCA studies performed were based on pilot-scale data, which cannot represent a real industrial production system. Therefore, Roibas-Rozas et al.[123] performed LCA for a mixed microbial culture-based full-scale PHA production using mussel cooking wastewater and compared it with

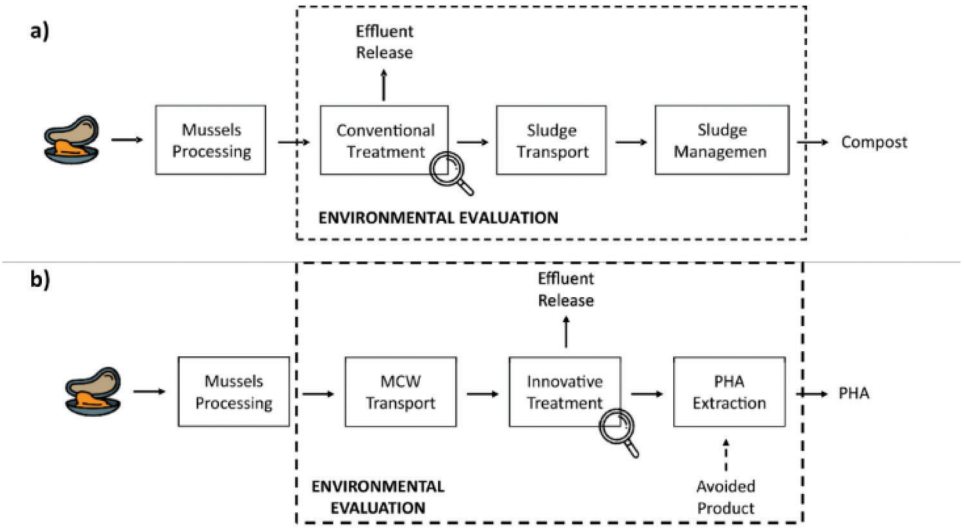

Figure 9. Scenario for a) linear economy, b) circular economy. The dashed-line square represents the system boundaries. Reproduced with permission.[123] Copyright 2021, Elsevier.

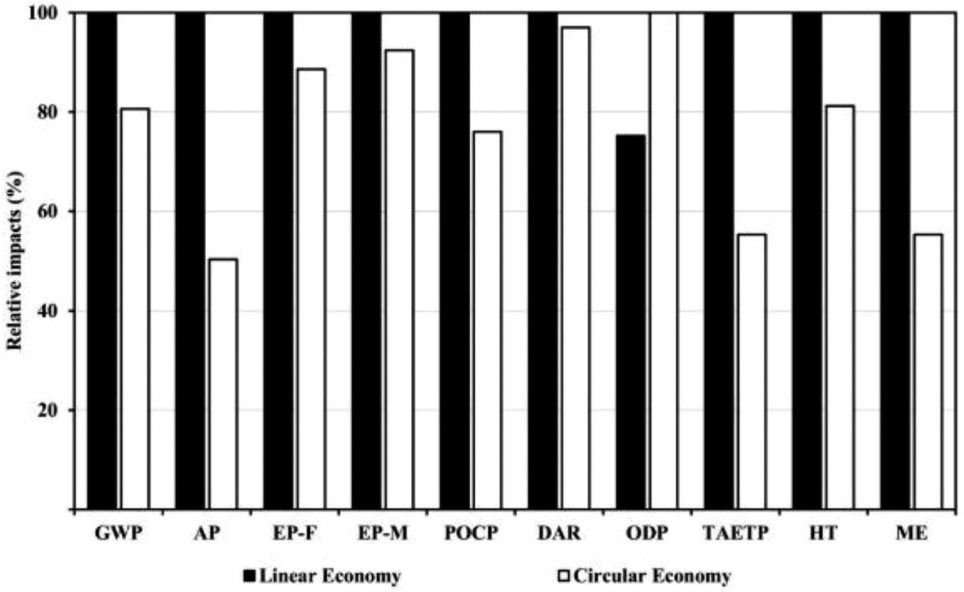

Figure 10. Comparison of linear and circular economy approach. These categories include GWP, AP, freshwater eutrophication potential (EP-F), marine eutrophication potential (EP-M), photochemical oxidant creation potential (POCP), DAR, ozone depletion potential (ODP), TAETP, HT, and ME. Reproduced with permission.[123] Copyright 2021, Elsevier.

conventional treatment processes. The different scenarios for linear economics (conventional method) and circular economy (invention method) are shown in **Figures 9** and **10**. The result revealed that the circular economy approach is better than the linear economy approach in all the impact categories evaluated, except the ozone depletion potential. In the linear economic approach, electricity highly influences (contributed between 12% and 68%) all environmental impact categories (except marine eutrophication potential). The chemicals consumption from the linear economic approach has a profound impact between 13% and 87% on human toxicity (HT), terrestrial, aquatic ecotoxicity potential (TAETP), and marine ecotoxicity (ME) and is mainly contributed by hydrochloric acid production. The most obvious environmental benefit associated with the circular economic approach (utilized wastewater) is in the category of fossil depletion (depletion of abiotic resources (DAR)) as the use of natural gas, and crude oil is prevented, as these are the raw materials for the production of plastic. Besides that, acidification potential (AP) and I were reduced by about 50% and 44%, respectively, as the formation of xylene was avoided in the circular economic pathway. The authors also proposed an integrated system by utilizing excess energy from the anaerobic digestion of waste sludge to maintain the temperature in the bioreactor and other downstream processes, saving ≈500 and 100 kWh per day, respectively.[123] The

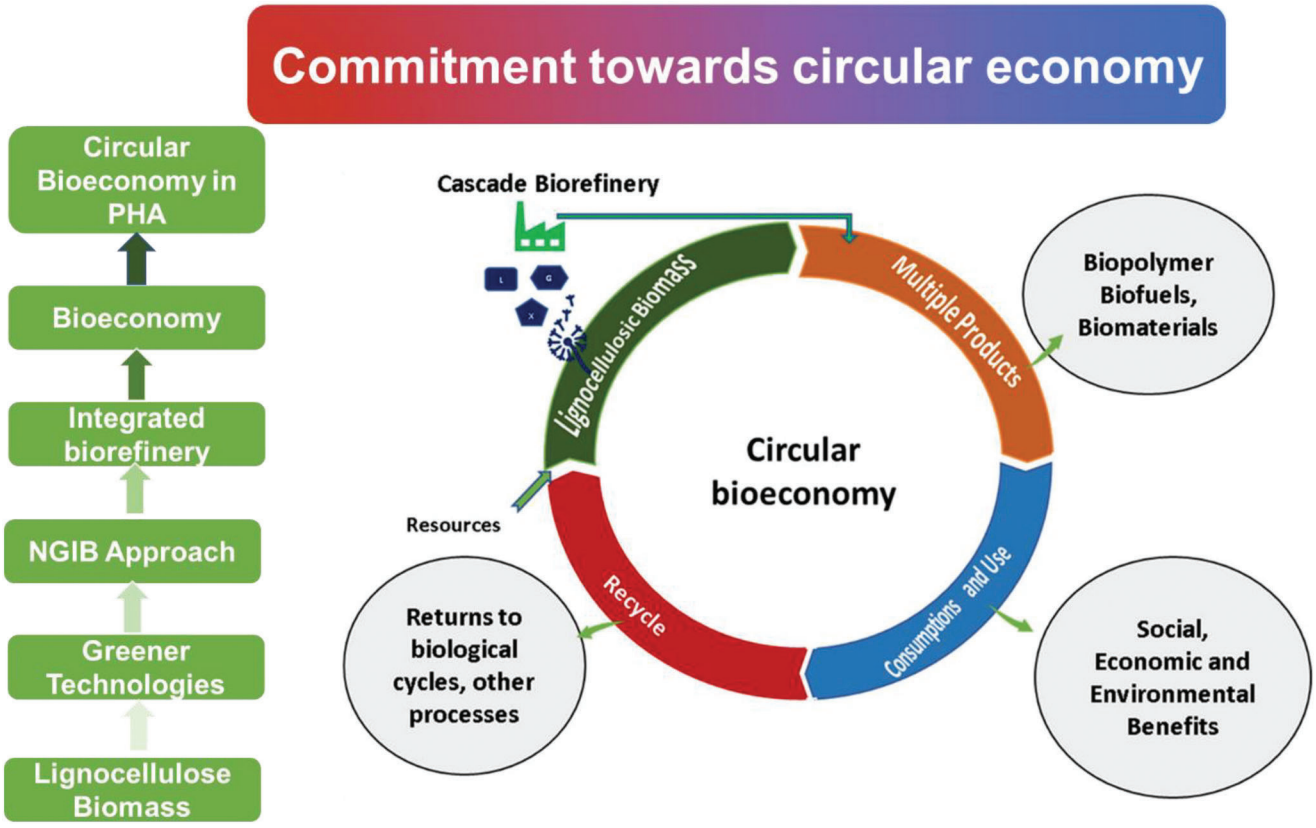

Figure 11. Illustration of the development of PHAs production toward the circular economy. The proposed closed-loop biorefinery design which fractionated all bio-based building blocks in biomass is converted into multispecialty products such as biofuels, PHAs biopolymer, and so on. The use and handling of these bioproducts in day-to-day life have social, economic, and environmental benefits. After handling these bioproducts at their end-life, they can be recycled via biological, chemical, and mechanical recycling processes.[130] Thus, minimizing waste emissions and promoting the circular economy in PHAs production. Adapted with permission.[37] Copyright 2022 American Chemical Society.

integrated approach proposed was reported to save about 15% of total energy consumption in the downstream processes. Unfortunately, the operation of the pilot-scale plant for PHAs production from the waste stream is limited worldwide, and the actual integration proposals are not well defined, so the potential environmental benefits remain unknown. Moreover, limited research is performed on the LCA between different downstream processes (e.g., extraction (solvent extraction, mechanical disruption), separation (precipitation, filtration), and purification (hypochlorite, ozone)). The environmental impact of these methods remains unknown, and the research gap needs to be filled to render high-value PHAs production from the waste stream more sustainable on a larger scale.

7. Progress of PHAs Production in the Context of the Circular Economy

The circular economy concept emphasizes the maximum recovery of products and minimum waste generation[39,124–126] and lignocellulosic biomass waste utilization in the biorefinery for the production of bio-based products is highly significant with concerning green chemistry principles.[127,128] The design of "cradle-to-cradle" biorefinery processes is essential to maintain the circular economy approach. The main goals of the circular economy are to recycle the end-life phases via biological and technical cycles and reuse them as a feedstock or starting material for another value chain, use renewable energy sources, and zero waste emission by modifying manufacturing processes and business models.[4,39,129–132] The current business model is modified to a sustainable manufacturing process, i.e., reuse, recycle, and reproduce to maintain the circular economy. PHAs' bioplastics are perfectly fit in this model since they are contributing to a more sustainable commercial life cycle by being part of a circular economy.[37] The progress of the lignocellulose-based PHAs production toward the circular economy is illustrated in **Figure 11**. The design of integrated processes for PHAs production within biorefinery is the paving pathway toward a sustainable circular economy. The life cycle and techno-economic assessment of the whole PHAs process are helping to maintain the circular economy by evaluating the environmental and economic performance of the design process. **Table 2** summarizes recent studies associated with biorefining pathways for the simultaneous production of PHAs and co-products and comments on maintaining the circular economy. The implementation of the integrated process requires technological advancement, economic investment, and financial subsidies provided by government agencies.

Table 2. Recent studies associated with integrated biorefinery approach for the production of PHAs and comments on the circular economy.

Biomass	Biorefining pathways	Impact categories	Comments on the circular economy	Ref.
Corn stover	- Plug-in processes (PIPOL) - Enzymatic hydrolysis - Fermentation	- Economic	- Integrated PIPOL design process promoting the circular economy by improving the cost of PHAs process and efficient valorization of lignin and carbohydrate sugars to PHA and cellulosic ethanol. - PHAs' costs improve as low as 6$ kg^{-1}, if the government provides subsidies, large-scale production of PHAs will be successful.	[35]
Giant cane	- ILs pretreatment - Enzymatic hydrolysis - Dark fermentation	- Economic	- Integrated approach to the production of PHAs, biohydrogen, and organic acid increased total revenue by fivefold and net revenue by tenfold as compared to the conventional approach. - The increased revenue helps to reduce the impact of biorefinery in economic terms.	[29]
Calophyllum inophyllum oilcake	- Ball milling - Fermentation	- Economic - Environmental	- Economic and environmental benefits increase with the simultaneous production of PHAs and biosurfactants. The proposed strategy is a viable solution to the development of sustainable and cost-effective lignocellulosic biorefinery.	[70]
Corn stover	- Acid pretreatment - Enzymatic hydrolysis - Alkaline treatment - Fermentation	- Environmental	- Co-fermentation of lignin and carbohydrate sugars boosts mcl-PHAs production. Valorization of lignin and sugars via an integrated approach promotes the circular economy in biorefinery	[133]
Corn cob	- Alkaline pretreatment - Enzymatic hydrolysis - Fermentation - Cell retention culture on membrane reactors	- Economic	Co-production of PHAs and astaxanthin using inexpensive and renewable corn cob demonstrate a reduction in high costs of PHAs and a positive impact on environmental sustainability	[134]
Urban waste	- Pretreatment with heterogenous catalyst - Phototrophic mixed culture	- Economic - Environmental	- Combining catalysts and biotechnological techniques via an integrated approach makes PHAs production more facile and efficient. The development of cost-efficient and environment-friendly heterogenous catalysts is important to boost biorefinery in the circular economy approach.	[135]

8. Current Challenges and Future Outlook

Lignocellulosic biomass represents the nonedible, abundant, carbon-neutral, and inexpensive raw material,[136] thus it has inspired various studies, aiming for the efficient utilization of it for PHAs production.[37,43,137,138] Lignocellulosic biorefinery processes biomass for PHAs production and is a highly promising and sustainable platform, however lignocellulosic biorefinery still faces some challenges such as costly biomass conversion process and low volume of PHAs production.[139]

Advances in biorefinery technologies have greatly contributed to the development of a new manufacturing concept for converting sustainable biomass into multiple products such as PHAs, biofuels, amino acids, proteins, and other fine chemicals, generally referred to as integrated lignocellulosic biorefinery. Metabolic engineering and synthetic biology tools and strategies play an important role to improve the production of PHAs through the development of high-performance strains for efficient utilization of renewable biomass. The consolidated bioprocessing approach, which combines biomass pretreatment with saccharification and fermentation is more beneficial for the production of PHAs and other value-added products.[51]

The realization of the circular economy approach in PHAs production recently become a hot topic in research.[37,130] If lignocellulosic biorefinery uses the advanced and green technologies mentioned above, it can help to mitigate the global environmental challenges due to the excessive use and production of fossil-based products. The circular economy model is based on the principle of reuse, reduce, and recycle, thus its implementation helps to achieve environmental sustainability and conserve natural resource for the next generation.[124] To assess better sustainability and economic viability of the biorefineries, advanced life cycle assessment and techno-economic tools are developed. These tools are important to assess the design biorefinery process for sustainability and economic viability before implementing it for large-scale PHAs production.

The scaling up of lignocellulose-based PHAs production is a very important topic for future research. The integration of biomass processing units, their optimization, technological innovation, development of robust microbial biocatalysts, risk assessment, and economic analysis are essential to transfer lab-scale PHAs production technology to real-world applications. However, several challenges remain in the development of greener technologies, robust strains, and discoveries covering the new biosynthetic, pathways toward realizing the integrated lignocellulosic biorefinery for sustainable and cost-efficient PHAs production. Nevertheless, considering that the integrated lignocellulosic biorefinery strategies improve the costs and yield PHAs production, diversified the products portfolio, and efficiently utilized the waste to upscale processes, the integrated biorefinery approach improves environmental sustainability and economic viability as compared to conventional biorefinery approach.

It is hoped that the key principles and strategies of consolidated bioprocessing, metabolic engineering, synthetic biology,

life cycle, and techno-economic provided in this review article to realize an integrated lignocellulosic biorefinery approach in PHAs production will be helpful for the researchers and industrialist who are interested in sustainable and cost-efficient biomass-based PHAs production.

9. Conclusions

Integration of the lignocellulosic biorefinery process improves PHA's production cost, diversifies the product portfolio, and efficiently valorizes waste to upscale processes. Integrating the process supports the production of value-added co-products along with PHAs., paving its path toward the circular economy by efficiently converting waste feedstock and minimizing waste generation. The efficient valorization of biomass needs to combine the upstream process with the downstream via an integrated biorefinery approach that helps to improve costs as well as enhance PHAs production. An integrated approach used to the processing biomass toward PHAs production pushed it to a circular economy concept, which makes the biorefinery process sustainable and economically viable. The future of PHAs production through an integrated biorefinery approach looks very promising in transforming PHAs products in the commercial market into the cost-competitive.

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Conflict of Interest

The authors declare no conflicts of interest.

Keywords

integrated biorefinery, life cycle assessment, polyhydroxyalkanoates, techno-economic analysis

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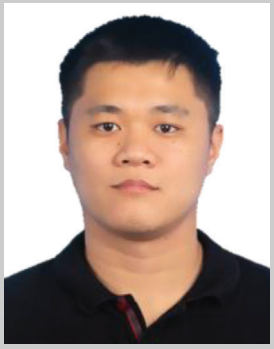
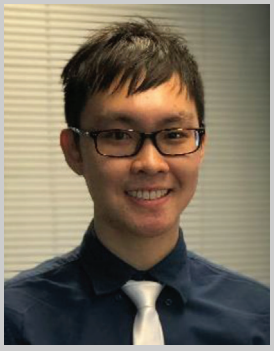
Vaibhav Vilas Andhalkar is a Ph.D. student at the Department of Chemical Engineering, Universitat Rovira i Virgili, Tarragona, Spain. He obtained his master's degree in chemistry from the University of Pune, India. He worked in the pharmaceutical and chemical industries for more than seven years. His research focused on the development of efficient and sustainable processes for the conversion of biomass to high-value-added products such as polyhydroxyalkanoates bioplastics, and platform chemicals. Currently, he has been working on biomass conversion via fermentation, bio-catalysis, and the development of circular bioeconomy processes.

Shin Ying Foong hails from Malaysia and is pursuing her Ph.D. at Universiti Malaysia Terengganu. Her research focuses primarily on thermochemical processes, waste valorization, and microwave technologies. With research experience in advanced pyrolysis technologies involving microwave, vacuum and CO_2 conditions, and co-pyrolysis, she aims to advance the understanding of pyrolysis processes in waste valorization. She believes that these efforts can provide viable alternatives to existing global issues and make a significant impact in the field of waste management. With her dedication and expertise, she aims to make a positive contribution to the scientific community.

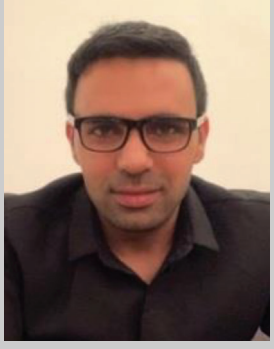


Seng Hon Kee graduated from Universiti Malaysia Terengganu with a bachelor's degree in marine biology in 2019. He is pursuing his doctorate in the same institution in microbial biotechnology. He currently works on production and characterization of rhamnolipids produced from carbon sources derived from microwave pyrolysis of medical plastic wastes. He has also worked on projects related to polyhydroxyalkanoate.

Su Shiung Lam is currently a professor and director of International Centre of Universiti Malaysia Terengganu. He leads a research group working mainly on chemical and environmental engineering, focusing on waste and biomass utilization, waste and wastewater treatment, green technology, and pollution mitigation. He is active in research on the application of thermochemical processes such as pyrolysis and microwave heating in transforming waste and biomass into green energy and products applicable to industry for carbon capture (e.g., CO_2 absorption), carbon emission reduction (e.g., gasification/pyrolysis of hydrocarbon feedstock, CO_2 utilization and conversion), and environmental protection.



Yi Herng Chan is currently a senior researcher and engineer working in the field of carbon capture and utilization (CCU) and hydrogen in PETRONAS Research Sdn. Bhd. (PRSB). He obtained his Ph.D. and M.Sc. in chemical engineering from Universiti Teknologi PETRONAS (UTP), Malaysia. His research interests include thermochemical conversions of wastes to H_2/syngas and value-added chemicals/products (pyrolysis, hydrothermal liquefaction, gasification), applications of supercritical fluids and life cycle assessment (LCA).



Ridha Djellabi is a researcher in the field of environmental remediation and energy production with international experience in different countries including Algeria, Italy, Portugal, China, and Spain. Currently, Ridha is a personal investigator at CATHETER-Universitat Rovira i Virgili since June 1, 2022 as he won Maria Zambrano Grants-2021 for international talents in Spain. Ridha does research in solar materials design for photocatalytic and photothermal applications. Ridha has published more than 100 scientific papers in relevant journals. He ran several special issues and he is associate editor at Frontiers.

Kesaven Bhubalan is an associate professor with the Faculty of Science and Marine Environment, Universiti Malaysia Terengganu. He obtained his bachelor's degree in marine biology from Kolej Universiti Sains dan Teknologi Malaysia in 2005 and Ph.D. degree in microbial biotechnology from Universiti Sains Malaysia in 2010. His field of expertise includes biomaterials, bioprocess, and biodegradation. He is actively engaged in research and has designed and performed experiments on bacteria-derived polyhydroxyalkanoate and rhamnolipid.

Francesc Medina obtained his Ph.D from the Universitat Rovira I Virgili (1993). In 2009, he received the ICREA Acadèmia award from the Generalitat de Catalunya, and in 2014, he was appointed as a 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) and de Innovation Center AMIC.

Magda Constantí graduated in biology (University of Barcelona, 1984) and did a master's degree in genetics in 1985. Her Ph.D. focused on the biodegradation of organic sulfur contained in coal in 1992. Afterward, she did several postdoctoral stays at the University of California, Los Angeles (USA), under the supervision of Dr. Robert P. Gunsalus performing genetic engineering. She also studied the microbiology of alcoholic and malolactic fermentations at University Rovira I Virgili (URV). Currently, she is an associate professor at the Chemical Engineering Department of URV, and her research focuses on the valorization of biomass to lactic acid, polyhydroxyalkanoates, and hydrogen.