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# Emerging approaches in lignocellulosic biomass pretreatment and anaerobic bioprocesses for sustainable biofuels production

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ABSTRACT

The development of advanced biofuels from waste organic matter, such as lignocellulosic biomass, is critical for global sustainable waste management and to delay climate change by reducing greenhouse gas emissions *via* partial replacement of fossil fuels. However, the inherent recalcitrance of lignocellulosic biomass due to the presence of inhibitory components, mainly lignin, limits the hydrolysis of its carbohydrate content, representing a key hurdle augmenting biofuel production. Therefore, pretreatment of lignocellulosic biomass is crucial to promote its fragmentation, increase its surface area and solubility, and lower the cellulose crystallinity and lignin content for sustainable biorefinery. Conventional pretreatment processes have several drawbacks, including high operational costs, corrosion of equipment, and generation of toxic effluents and by-products. To offset the negative impacts of these limitations on biofuel production, here, we have discussed and critically compared various eco-friendly approaches for the efficient conversion of biomass to ensure high yields of biofuels as a commercial solution. Moreover, a range of microbes and enzymes have been highlighted that effectively utilize lignocellulosic biomass to obtain energy and convert its complex polymeric structure into a biodegradable one, facilitating its subsequent valorization. Furthermore, the importance of multi-omics approaches was discussed to gain functional insights into the lignocellulolytic microbial communities and their interspecies symbiosis during the hydrolysis of organic biomass. Finally, the limitations of previous studies, challenges, industrial perspectives, and future outlooks for the development of economical, energy-saving, and eco-friendly strategies toward the sustainable valorization of lignocellulosic biomass were summarized.

## 1. Introduction

The bioeconomy of the 21st century has encouraged the development of novel economic models to curtail the utilization of natural resources for economic prosperity and ecological survival (Hassan et al., 2018; Kumar et al., 2020a). Global warming and climate change, environmental deterioration, and consequent increase in pollution-related health hazards are significant threats due to the ever-growing demand and consumption of fossil fuels (Kazemi Shariat Panahi et al., 2019). Intense efforts are being made to channelize sustainable energy from the largest renewable carbon source, the lignocellulosic biomass (LCB), which is an attractive feedstock for anaerobic bioprocessing (Cheng et al., 2020). This will help to meet the global energy demand, reduce the dependence on fossil fuels, and reduce greenhouse gas (GHG) emissions by 20–70%, thereby encouraging sustainable green growth (Xu et al., 2016).

Biorefineries can be used to produce energy, value-added chemicals, bioplastics, and fuels using renewable and non-edible feedstocks, such as LCB and food waste biomass, to drive society towards a sustainable economy (Sriariyanun et al., 2021). However, numerous challenges, such as the presence of inhibitory molecules and the structural complexity of LCB due to cross-linking between polysaccharides (cellulose and hemicellulose) and phenolic polymers (lignin), critically

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**Abbreviations**

| | |
|---|---|
| ABE | Acetone-butanol-ethanol |
| AD | Anaerobic digestion |
| APSs | Atmospheric plasma sources |
| BSG | Brewer's spent grain |
| CAZymes | Carbohydrate-active enzymes |
| CBP | Consolidated bioprocessing |
| CS | Cornstalk |
| DES | Deep eutectic solvent |
| DP | Degree of polymerization |
| EB | Electron beam |
| EU | European Union |
| FTIR | Fourier transform infrared |
| GC-MS | Gas chromatography-mass spectrometry |
| HTC | Hydrothermal carbonization |
| GHG | Greenhouse gas |
| 5-HMF | 5-hydroxymethyl furfural |
| IL | Ionic liquid |
| iTRAQ | isobaric tagging approach for relative and absolute quantification |
| LCA | Life-cycle assessment |
| LCB | Lignocellulosic biomass |
| LCC | Lignin carbohydrate complex |
| LC-MS | Liquid chromatography-mass spectrometry |
| LC-MS/MS | Liquid chromatography-tandem mass spectrometry |
| LPMOs | Lytic polysaccharide monooxygenases |
| MWI | Microwave irradiation |
| NGS | Next-generation sequencing |
| NIMS | Nanostructure initiator mass spectrometry |
| OUT | Operational taxonomic unit |
| PE | Plasma electrolysis |
| PEF | Pulse-electric field |
| PNS | Purple non-sulfur |
| PUL | Polysaccharide utilization loci |
| SC-$CO_2$ | Supercritical carbon dioxide |
| SEM | Scanning electron microscopy |
| SEP | Steam explosion pretreatment |
| SHF | Separate hydrolysis and fermentation |
| SSCF | Simultaneous saccharification and simultaneous saccharification and co-fermentation |
| SSF | Simultaneous saccharification and fermentation |
| TRS | Total reducing sugar |
| TVS | Total volatile solids |
| MC1 | Thermophilic microbial consortium |

reduce access to the carbohydrate content (55–75% w/w); therefore, it is necessary to develop effective anaerobic bioprocessing methods towards a green environment and to boost the economy (Vu et al., 2020). Understanding the nature and chemical composition of feedstock and appropriate biological and non-biological (single or combinations of physical, chemical, physicochemical methods) pretreatment technologies are necessary for the effective valorization of LCBs into biofuels (Saravanakumar and Kathiresan, 2014).

Economic assessments have shown that the pretreatment step of LCB is the most energy-intensive step and can account for 40% of the total processing cost, which often limits the commercial utilization of LCBs for biofuel production. In addition, most of the physicochemical pretreatment processes intended to improve the biodegradability of LCB simultaneously release inhibitory byproducts that adversely affect the microbial growth and functions during anaerobic bioprocesses (Ravindran and Jaiswal, 2016). In this regard, cost-effective and eco-friendly pretreatment with lignocellulolytic bacteria and fungi (primarily white-rot fungi) or their enzymes is an efficient biological process, which can be completed within a few hours to several days without generating inhibitory compounds, resulting in a significant increase in the biofuel yield (up to 120%) (Zabed et al., 2019). However, a long pretreatment time, relatively low hydrolysis rate, and considerable loss of fermentable sugars are some of the bottlenecks associated with the use of microbial pretreatment methods (Basak et al., 2020b).

Various eco-friendly, efficient, and cost-effective pretreatment methods, including the use of ultrasound, microwaves, supercritical carbon dioxide (SC–$CO_2$), deep eutectic solvents (DESs), and ionic liquids (ILs), are the emerging physicochemical pretreatment methods for LCB valorization. These emerging technologies (biological and non-biological) can be used as commercially feasible green pretreatment methods for the sustainable and efficient pretreatment of LCB (Rahmati et al., 2020). Further, it is essential to identify the key microbial players and the metabolic pathways involved in hydrolysis during biological pretreatment *via* a combination of 'omics' approaches, which will aid in designing an improved microbial/enzymatic cocktail for effective LCB degradation (Alessi et al., 2018; Tsapekos et al., 2017). Biorefining of pretreated biomass *via* anaerobic bioprocesses involves fermentative anaerobes (obligate or facultative) that produce hydrogen ($H_2$), methane ($CH_4$), ethanol, butanol, and acetone as one of their metabolic end products (Soares et al., 2020). Anaerobic digestion (AD) and dark fermentation are the most reliable and efficient processes for waste-to-energy conversions (Basak et al., 2020a; Mulat et al., 2018); however, these complex processes involve various hydrolytic, fermentative, syntrophic, and methanogenic archaea bacteria in an anoxic environment to form bio-methane/$H_2$ from organic waste.

Many pretreatment strategies have been practiced for decades to improve the fermentability of biomass by altering its physical and chemical structure (Saha et al., 2016). However, techno-economic feasibility, carbon footprints, slow process, and generation of several inhibitory by-products are the major concerns of the conventional pretreatment processes for the subsequent valorization of LCB (Ab Rasid et al., 2021; Vu et al., 2020). Considering these, the current article reviews and critically analyzes the recent developments and optimizations from the perspective of sustainable valorization of LCB for biofuels production. Several emerging pretreatment processes, generation of inhibitory by-products and their detoxification, and their effects on the valorization of LCB have been discussed here, which offer possible solutions in biorefineries for higher productivity, substrate efficiency, reducing toxicity and waste generation, economical, and sustainable manufacturing processes. Besides, accelerated degradation of LCB has also been highlighted by using enzymatic machinery of ruminant anaerobic, potent lignocellulolytic bacteria and fungi to enhance the rate-limiting hydrolytic pathway for smooth conversion of LCB into bioenergy.

A contemporary insight on 'multi-omics' approach has also been discussed for the first time to unravel the structural and functional understandings into the lignocellulolytic microbes and microbial communities that can enhance LCB decomposition and improve the efficiency of biofuel production. Furthermore, the significant findings of previous studies on the production of bioenergy using a lignocellulosic substrate, with a specific focus on the relevant anaerobic bioprocesses, such as AD, photo/dark-fermentation, ethanol fermentation, and acetone-butanol-ethanol (ABE) fermentation, were outlined. We performed a comprehensive literature survey on various methods for the pretreatment and valorization of LCB and sustainable and economical treatment approaches, emphasizing feasible concepts, desirable strategies, and practical aspects for the commercial success of such anaerobic bioprocesses. The review articles published over the last five years in the

relevant domain are tabulated and compared in Table 1 to validate the novelty of the present work.

## 2. Structural complexity of LCB

### 2.1. Chemical and physical complexities of LCB

Cellulose constitutes the basic structure of LCB and imparts rigidity and ductility. This polymer includes repeated units (400–1400) of $\beta$-D-glucopyranose linked by $\beta$-(1–4) glycosidic bonds (Fig. 1), which are mostly crystalline fibril structures embedded in LCB that confer resistance to biodegradation (Zeng et al., 2014). The degree of polymerization (DP) of cellulose plays a crucial role in maintaining the rigid structure of LCBs as long chains of cellulose contain many intermolecular H-bonds in-between the '-OH' group of C3 position and 'O' of glycosidic ring, which confer high resistance against its biodegradation. The accessibility and reactivity of the functional groups present in cellulose molecules could be affected by an alternation of its crystalline and amorphous regions. The macromolecules in the amorphous structure of cellulose are irregular and wide apart, resulting in a lower density of 1.5 g/cm$^3$ compared to 1.588 g/cm$^3$ in crystalline structure due to the regular and compact arrangement of macromolecules (Bonechi et al., 2017).

Hemicellulose, a complex and branched heterogeneous polymer, is composed of pentoses (D-xylose, L-arabinose) and hexoses (D-glucose, D-mannose, D-galactose) (Chandel et al., 2018). In addition, a trace amount of L-rhamnose, D-glucuronic acid, and D-galacturonic acid are complexly linked to each other (Hendriks and Zeeman, 2009). However, the chemical composition, DP, and branch-chain composition of hemicellulose differ according to the cell type and plant species. The main component of hemicellulose in grass species is glucuronoarabinoxylans, while galactoglucomannan and glucuronoxylan are primarily present in softwood and hardwood along with other hemicelluloses (Scheller and Ulvskov, 2010). Hemicelluloses in many species are acetylated xylan and mannan units to various degrees at C2 or C3 position, especially in dicot secondary walls and grasses have ferulic acid in xylans, which is covalently linked to *p*-coumaric acid of lignin (Ebringerová et al., 2005; Hatfield et al., 2008). Hemicellulose has an amorphous structure with a DP of approximately 100–200 units lower than that of cellulose. Despite its weak physical strength, it strongly limits cellulase activity (Barhoum et al., 2020).

Lignin is the second most widely distributed terrestrial biopolymer after cellulose in biomass, used for binding and putting the fibers together to give a compact and resistant plant cell structure (Boerjan et al., 2003). It consists of three 4-hydroxycinnamyl alcohols representing basic aromatic moieties, such as *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) which formed macromolecule through radical oxidative polymerization (Tursi, 2019). The DP for the aromatic polymer varies from 450 to 550 units, which holds the hemicellulose and cellulose fibers together in the plant cell wall and provides structural rigidity (Zoghlami and Paes, 2019). Several G and S units with a small number of H units are present in hardwood lignin, while G units predominate the others in softwood lignin. Herbaceous lignin contains all three types of monolignols in varying amounts (Zhao et al., 2012). Lignin composition (i.e., the number of hydroxyl groups and S and G units) and its content adversely affect the release of cellulose during the hydrolysis of LCB. Hence, the elimination of lignin from LCB disrupts the lignin carbohydrate complex (LCC), thereby enhancing the porosity, surface area, and solubility of the biomass (Karimi and Taherzadeh, 2016; Kruyeniski et al., 2019). In addition to the three polymers described above, many other components are also included in the biomass (referred to as extractives, presenting <10% w/w of the biomass), which prevent the penetration of microorganisms into the plant (Mcdonough, 1983).

### 2.2. Complexity of valorizing LCB in anaerobic bioprocessing

The diversity of raw materials presents in the biomass, which includes various plant groups, their different chemical compositions, and physical properties, make it difficult to scale up and commercialize pre-processing at the industrial level to produce bioenergy. The effective use of hemicellulose (which contributes 40–50% of the total carbohydrate content) and cellulose is crucial to obtaining high yields and productivity during anaerobic bioprocesses. Although hemicellulose is sensitive to water and acid treatment and is easily converted to monosaccharides, its tight binding to cellulose *via* H-bonds and covalent linkage with lignin to form LCC affects its hydrolysis. It renders the LCB recalcitrant to hydrolysis and necessitates pretreatment to make the structure porous and alter the structural and compositional rigidity priors to its valorization (Dahiya et al., 2018). The total reducing sugar (TRS) yield from LCB can be enhanced from 10%–90% with an appropriate pretreatment method following enzymatic saccharification (Zhang et al., 2012).

In general, depending on the severity of the process conditions, such as the chemical pretreatments using various acids, pentoses present in hemicellulose may be over-degraded to form furfural and organic acids, which in turn inhibit microbial action during AD (Basak et al., 2020a). In particular, phenolic compounds produced during the decomposition of lignin polymers display potent inhibitory or toxic properties against microorganisms, which makes biological conversion *via* saccharification and fermentation challenging task. Lignin also shows adverse effects by blocking enzyme accessibility to cellulose and non-specifically adsorbing hydrolytic enzymes to its sticky surface results in non-productive binding of cellulolytic enzymes to LCC (dos Santos et al., 2019). Therefore, removing or reducing the lignin content in LCB is necessary by selecting an appropriate and effective pretreatment method. Moreover, various intrinsic characteristics, such as dielectric properties, number of hydroxyl groups, moisture content, and crystalline/amorphous region in the LCB, should be considered while designing the parametric conditions. Adopting a strategy for maximal utilization of the monomeric sugars produced in LCB can overcome complexity and valorize LCB in anaerobic bioprocessing.

## 3. Emerging pretreatment technologies for LCB

Conventional pretreatment methods remain insufficient to meet the requirements for industrial-scale biofuel production due to their poor techno-economic and environmental sustainability. The emerging physicochemical and biological pretreatment methods have been discussed here while highlighting the current findings and recent innovations to offset the inherent challenges to their applications in commercial-scale biorefinery processes.

### 3.1. Emerging non-biological pretreatment methods

#### 3.1.1. Physicochemical pretreatment methods

Several promising and non-classical technologies, including the use of microwaves, ultrasound, radiation, steam explosion pretreatment (SEP), hydrothermal, and pulse-electric field (PEF), have been proposed as commercial solutions for LCB valorization (Fig. 2). Non-conventional heating sources, such as microwave irradiation (MWI) operated under atmospheric or high pressure can exert effects at the molecular level and dissipate uniformly and more rapidly, resulting in increased surface area due to swelling, fragmentation, and internal chemical changes (decarboxylation and dehydration) (Dai et al., 2017). However, the efficiency of MWI depends on the dielectric properties of feedstocks, which reflect the capacity of the substrate to store electromagnetic energy and convert it into heat. High moisture and inorganic content in the carbon-rich materials are better microwave energy absorbers and quickly achieve the hydrothermal conditions required for the hydrolysis of LCB (Li et al., 2016). However, MWI was ineffective on the plant fiber material under low temperature (<100 °C) (Chen et al., 2017). Additionally, high

**Table 1**
Comparative analysis of selected review articles published over the last five years on the valorization of lignocellulosic biomass for biofuels production.

| Ref. | Highlights and strength of the review | Review coverage relevant to the sustainable valorization of LCB for biofuels production | | | | | |
|---|---|---|---|---|---|---|---|
| | | Structural complexity of LCB | Biomass pretreatment methods | Generation of inhibitors and their detoxification | Multi-omics approach | Anaerobic bioprocesses for biofuels production | Sustainability aspects |
| Kumar and Sharma (2017) | - Mainly discussed the pretreatment methods for LCB | N.C. | - Physical, chemical, physicochemical, and biological methods | N.C. | N.C. | - Briefly mentioned the biofuels and bioproducts production | N.C. |
| Chandel et al. (2018) | - Commercialization of cellulosic ethanol and biochemical products are mentioned<br>- Biomass supply chain, processing, and product recovery have been presented | N.C. | - Enzymatic hydrolysis | N.C. | N.C. | - General biorefinery | N.C. |
| Hassan et al. (2018) | - Discussed the limitation of conventional pretreatment of LCB<br>- Focused on the green approaches of pretreatment<br>- Techno-economic feasibility studies are shown | N.C. | - Physicochemical pretreatment | N.C. | N.C. | N.C. | N.C. |
| Baruah et al. (2018) | - Critically discussed the currently used pretreatment strategies with merits and demerits<br>- Recovery of high-value bio-polymeric components from LCB is mentioned | - Covered | - Physical, chemical, physicochemical, and biological pretreatment methods | N.C. | N.C. | N.C. | N.C. |
| Kumari and Singh (2018) | - Different generations of biofuels and their importance are shown | N.C. | - Conventional and emerging pretreatment methods | N.C. | N.C. | - Briefly discuss biofuels (biogas) production | N.C. |
| Hernández-Beltrán et al. (2019) | - Efficiency and limitations of various pretreatment strategies are shown<br>- Challenges and opportunities to increase biogas yield from LCB are reviewed | - Covered | - Conventional and emerging physicochemical and biological pretreatment methods | N.C. | N.C. | - AD for biogas production | - Briefly covered environmental aspects |
| Galbe and Wallberg (2019) | - Pretreatment of different lignocellulosic materials are shown<br>- Mentioned the techno-economic feasibility studies of LCB biorefinery | N.C. | - Covered conventional pretreatment methods | N.C. | N.C. | - Biorefinery of LCB | - LCA of LCB biorefinery |
| Tu and Hallett (2019) | - Discussed the recent advances in pretreatment methods of LCB | N.C. | - Physical, chemical, physicochemical, and biological pretreatment methods | N.C. | N.C. | N.C. | N.C. |
| Zabed et al. (2019) | - Mechanism and factors affecting the biological pretreatment methods are given<br>- Discussed the limitation of | - Briefly covered | - Recent advances in biological pretreatment methods | N.C. | N.C. | N.C. | N.C. |

(continued on next page)

**Table 1** (*continued*)

| Ref. | Highlights and strength of the review | Review coverage relevant to the sustainable valorization of LCB for biofuels production | | | | | |
|---|---|---|---|---|---|---|---|
| | | Structural complexity of LCB | Biomass pretreatment methods | Generation of inhibitors and their detoxification | Multi-omics approach | Anaerobic bioprocesses for biofuels production | Sustainability aspects |
| | physicochemical pretreatments<br>- 2G and 3G biofuels production are reviewed using LCB and microalgae | | | | | | |
| Bhatia et al. (2020) | - Technological improvement, challenges, and key parameters of pretreatment of LCB are given | - Covered | - Recently developed physicochemical pretreatment methods | - Covered | N.C. | N.C. | N.C. |
| Rahmati et al. (2020) | - Discussed the pretreatment and fermentation of LCB<br>- Mainly focused on reaction mechanism and process engineering | N.C. | - Physical, chemical, physicochemical, and biological pretreatments methods | N.C. | N.C. | N.C. | N.C. |
| Kumar et al. (2020c) | - Mechanisms, merits, and demerits of different pretreatment methods for biorefinery of LCB are given | - Covered | - Physical, chemical, physicochemical, and biological pretreatment methods | N.C. | N.C. | - Briefly discussed the different generations of biofuels | - Economic aspects of pretreatment |
| Haldar and Purkait (2021) | - Insight of reaction mechanism, challenges, and perspectives of emerging pretreatment methods are discussed | - Covered | - Emerging physicochemical pretreatment methods | N.C. | N.C. | N.C. | N.C. |
| Mankar et al. (2021) | - Recent advances and future perspectives of physical and chemical pretreatment methods are discussed | - Covered | - Conventional and emerging physicochemical pretreatment methods | N.C. | N.C. | N.C. | N.C. |
| Beig et al. (2021) | - Challenges and innovative developments in LCB pretreatment for biofuels production are critically mentioned | N.C. | - Physical, chemical, physicochemical, and biological pretreatment methods | N.C. | N.C. | N.C. | - LCA of pretreatment methods |
| Ab Rasid et al. (2021) | - Recent advances and future perspectives in green pretreatment methods of LCB for enhanced biofuels production are discussed | N.C. | - Green chemical pretreatment methods | N.C. | N.C. | - AD for methane, bioethanol, and biobutanol fermentations | N.C. |
| Mirmohamadsadeghi et al. (2021) | - LCB pretreatment and AD improvement mechanisms are reviewed<br>- Microbial diversity during AD is discussed | - Covered | N.C. | - Covered | - Phylogenetic studies | - AD for biogas production | N.C. |
| Culaba et al. (2022) | - Challenges and future research about innovative approaches, such as the use of artificial intelligence in lignocellulosic | N.C. | - Briefly covered pretreatment | N.C. | N.C. | - Briefly mentioned the production of value-added products from LCB | - CE concept in smart biorefinery |

(*continued on next page*)

**Table 1** (*continued*)

| Ref. | Highlights and strength of the review | Review coverage relevant to the sustainable valorization of LCB for biofuels production | | | | | |
|---|---|---|---|---|---|---|---|
| | | Structural complexity of LCB | Biomass pretreatment methods | Generation of inhibitors and their detoxification | Multi-omics approach | Anaerobic bioprocesses for biofuels production | Sustainability aspects |
| | biorefinery, are discussed | | | | | | |
| Ashokkumar et al. (2022) | - Advanced pretreatment methods of LCB for biofuels and bioproducts production are mentioned | - Covered | - Conventional and emerging physicochemical and biological pretreatment methods (briefly discussed) | N.C. | N.C. | - Briefly mentioned the production of biofuels and value-added products | - Techno-economic feasibility of biomass valorization |
| Rodionova et al. (2021) | - Comprehensively discussed the lignocellulosic biorefinery<br>- Biochemical conversion and engineering perspective of LCB biofuels are mentioned | - Covered | - Briefly Covered the physical, chemical, and biological pretreatment | N.C. | N.C. | - Briefly Covered bio-$H_2$, bioethanol, and bio-butanol | N.C. |
| Zhao et al. (2022) | - Advances, key challenges, and perspective LCB pretreatment for bioenergy production are discussed | N.C. | - Conventional and emerging physical, chemical, physicochemical, and biological pretreatment methods | - Covered | N.C. | - Briefly mentioned bioenergy production in general | N.C. |
| Present study | - Critically discussed the emerging pretreatment methods of LCB<br>- Sustainable valorization of pretreated LCB using anaerobic bioprocesses is reviewed | - Covered in detail about the complexity of different components of biomass | - Different emerging physicochemical, chemical, and biological pretreatment methods | - Inhibitors generations, their effect on the valorization of LCB, and detoxification methods | - Discussed genomics, transcriptomics, proteomics, and metabolomics | - Different anaerobic bioprocesses, such as light/dark fermentation, AD, bioethanol, and ABE fermentation | - Discussed sustainability aspects of valorization of LCB |

'N.C. - Not covered'.

capital and operating costs, energy-intensive processes, and biomass with high moisture and inorganic contents should be optimized before implementing MWI on an industrial scale. Ultrasound pretreatment in the range of 20 kHz to 1 MHz has been used to produce oxidizing radicals that attack and disrupt the linkages ($\alpha$-O-4 and $\beta$-O-4) present in the lignin of LCB, leading to the fragmentation, rupture, and destruction of cells (Luo et al., 2013).

Ultrasound-assisted treatment results in the development of cavitation bubbles inside the LCB, which subsequently collapse after attaining the critical size owing to the development of high pressure (182.3 MPa) and temperature (1726–4726 °C). This leads to the splitting of the hemicellulose and cellulose structure, as well as fractionation in the lignin polymers (Kumar and Sharma, 2017). The addition of ammonia (10 mL/g LCB) along with ultrasound treatment (400 W and 24 kHz) resulted in >58% delignification and >95% cellulose recovery from sugarcane bagasse at 80 °C for 45 min (Ramadoss and Muthukumar, 2014). Thus, the synergistic approach reduced by-product formation, operating temperature, pretreatment time, and cellulose crystallinity. Despite several advantages, to augment the bioconversion of LCB *via* ultrasound processes at an industrial scale, various factors must be critically comprehended and optimized, such as operating time, ultrasound frequency, solvents, type of feedstock, and bench-scale results (Mankar et al., 2021).

Radiations from different radioisotopes, such as Co-60 and Cs-137, can penetrate deeply and form free radicals to alter lignin structure, reduce cellulose crystallinity, and enhance specific surface area (Hong et al., 2014). Similarly, irradiation by an electron beam (EB) obtained from a linear accelerator produces free radicals and can disrupt the cell wall structure by chain scission or cross-link formation, depolymerization, and decrystallization of biomass (Schnabel et al., 2015). However, additional methods, such as a steam explosion or alkali treatment along with EB, are required to effectively depolymerize LCB (Xiang et al., 2017).

Aqueous fractionation technologies include SEP (Pielhop et al., 2016) and hydrothermal (Xiao et al., 2014) methods. Here, water is used at high temperatures (160–240 °C) and pressure (0.7–4.8 MPa) for hydrolysis and lignin separation, which renders the cellulose amenable to further enzymatic hydrolysis with a low effort for detoxification due to reduced generation of toxic compounds. Al Ramahi et al. (2021) reported hydrothermal carbonization (HTC) pretreatment of dried dairy sludge at 210 °C for 30 min to improve the methane yield up to 192% by 30% increase in sludge biodegradability and an 18% reduction in chemical oxygen demand through AD process. Further, the application of HTC of AD digestate enhanced the total energy production by forming hydrochar of a high calorific valued (10.2 MJ/kg) product. These methods do not require extra chemicals, thereby lowering the capital and operating costs, although they corrode the equipment slightly (Zhuang et al., 2016). Nevertheless, SEP and hydrothermal methods have been successfully applied in semi-industrial projects on a pilot scale, which indicates their potential for implementation at full industrial scale (Larsen et al., 2008; Thomsen et al., 2008). The PEF is a non-thermal and energy-efficient method that can disrupt the LCB structure by applying voltage pulses of 0.1–80 kV/cm for a fraction of time, facilitating the saccharification of porous biomass by hydrolytic enzymes (Hassan et al., 2018). However, high equipment cost, non-uniform effect, non-suitability for solid biomass, and operational

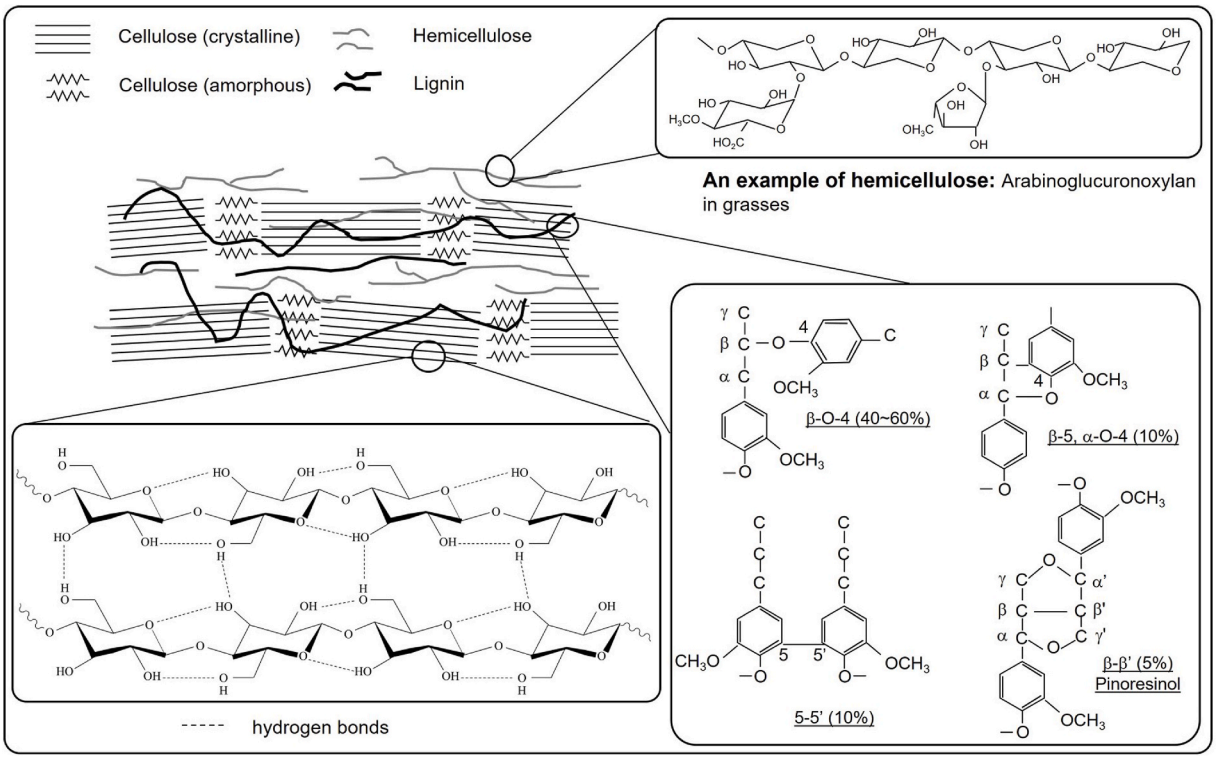


**Fig. 1.** Structural complexities of lignocellulosic biomass consisting of cellulose, hemicellulose, and lignin.

and safety issues are significant limitations of PEF-based LCB pretreatment (Kovacic et al., 2021).

### 3.1.2. *Emerging chemical pretreatment methods*

The idea of 'green chemistry' application in biorefineries for efficient conversion of LCB to biofuels without using harsh and toxic chemicals has aroused increasing interest in researchers (Yiin et al., 2021). Recently, advances in chemistry have led to the development of greener and more sustainable alternatives, such as ILs, DESs, SC-$CO_2$ explosion, and ozonolysis. ILs and DESs are the emerging, and tailor-made green solvents whose physicochemical characteristics (*viz.* polarity, viscosity, conductivity, hydrophobicity) can be tailored by adding various combinations of cationic and anionic constituents (Cao et al., 2017). ILs, such as [1-butyl, 3-methylimidazolium][hydrogen-$SO_4$] and [1-butyl, 3-methylimidazolium] [methyl-$SO_4$] have been used to fractionate LCB (*Pinus sylvestris* and *Miscanthus giganteus*) into lignin and hemicellulose in hydrolysate and cellulose as solid biomass (Brandt et al., 2011). High-purity cellulose can be recovered by adding an anti-solvent, such as aqueous ethanol or acetone, while ILs can be recovered *via* ion exchange, salting out, reverse osmosis or pervaporation. However, a few limitations are associated with ILs; pyridinium and imidazolium salts, mainly used in ILs, are obtained from a petroleum source, are costly, cause water pollution, and are non-biodegradable (Kumar et al., 2020b). Unlike ILs, DESs are eco-friendly, cost-effective, highly tunable, and soluble and render the LCBs amenable for better conversion to value-added products (Chen and Mu, 2019).

Another advanced chemical pretreatment is ozonolysis, where ozone, a powerful oxidizing agent, reacts specifically with the lignin of LCB, causing up to 80% delignification, resulting in enhanced LCB digestibility during the subsequent saccharification step (Rahmati et al., 2020). However, the high operating cost of ozone generation and limitations related to scaling up are a few significant constraints related to ozonolysis. In supercritical $CO_2$ (SC–$CO_2$) pretreatment performed under high pressure (14–18 MPa) at mild temperatures (30–100 °C), $CO_2$ molecules penetrate the crystalline structure of the LCB by forming carbonic acid and increasing the surface area (Garver and Liu, 2014). However, the efficacy of SC-$CO_2$ pretreatment heavily depends on the moisture content of the biomass because the $CO_2$ and moisture generate carbonic acids *in situ*, which promote hemicellulose hydrolysis due to acidification of the reaction mixture (Escobar et al., 2020; Fockink et al., 2018). The use of SC-$CO_2$ does not cause equipment corrosion, as by mineral acids, during industrial-scale applications because the pH of the residual solution increases after depressurization of $CO_2$ gas (Toscan et al., 2017).

Plasma pretreatment technology is another emerging efficient method being considered for inducing physical and chemical structural changes in LCB using highly reactive ionized gases without generating polluting and toxic chemicals (Vanneste et al., 2017). Variants of plasma pretreatment include atmospheric plasma sources (APSs) and plasma electrolysis (PE), which employ low gas input and electricity to generate powerful oxidizing molecules, such as hydroxyl radicals, singlet oxygen, and ozone to decompose/reduce the lignin content, increase the surface area, and reduce the crystallinity index by facilitating the severe collision of active species over the surface of biomass (Gao et al., 2014). However, initial moisture content of 50% was suitable for maximum delignification and cellulose recovery by ozonolysis through APS and PE processes (Tian et al., 2013a). The methods mentioned above are energy-intensive, whereas a renewable energy source can make the processes eco-friendly and sustainable (Table 2). Furthermore, deconstruction of lignocellulosic substrates into fermentable sugars becomes more effective when green pretreatment technologies (as discussed above) are performed in combination with integrated methods, as shown in Table 3.

### 3.1.3. *Generation of inhibitory by-products and their detoxification*

Pretreatment of LCB under thermal or thermochemical regimes results in better digestibility of lignocellulosic feedstocks, which also generates toxic by-products (Basak et al., 2020a). In oxidative pretreatment, hydrothermal pretreatment, and SEP, lignin undergoes depolymerization to form phenolic compounds (e.g., vanillin and syringaldehyde), whereas carbohydrates (cellulose and hemicellulose) transform into furanic compounds [furfural, levulinic acid,

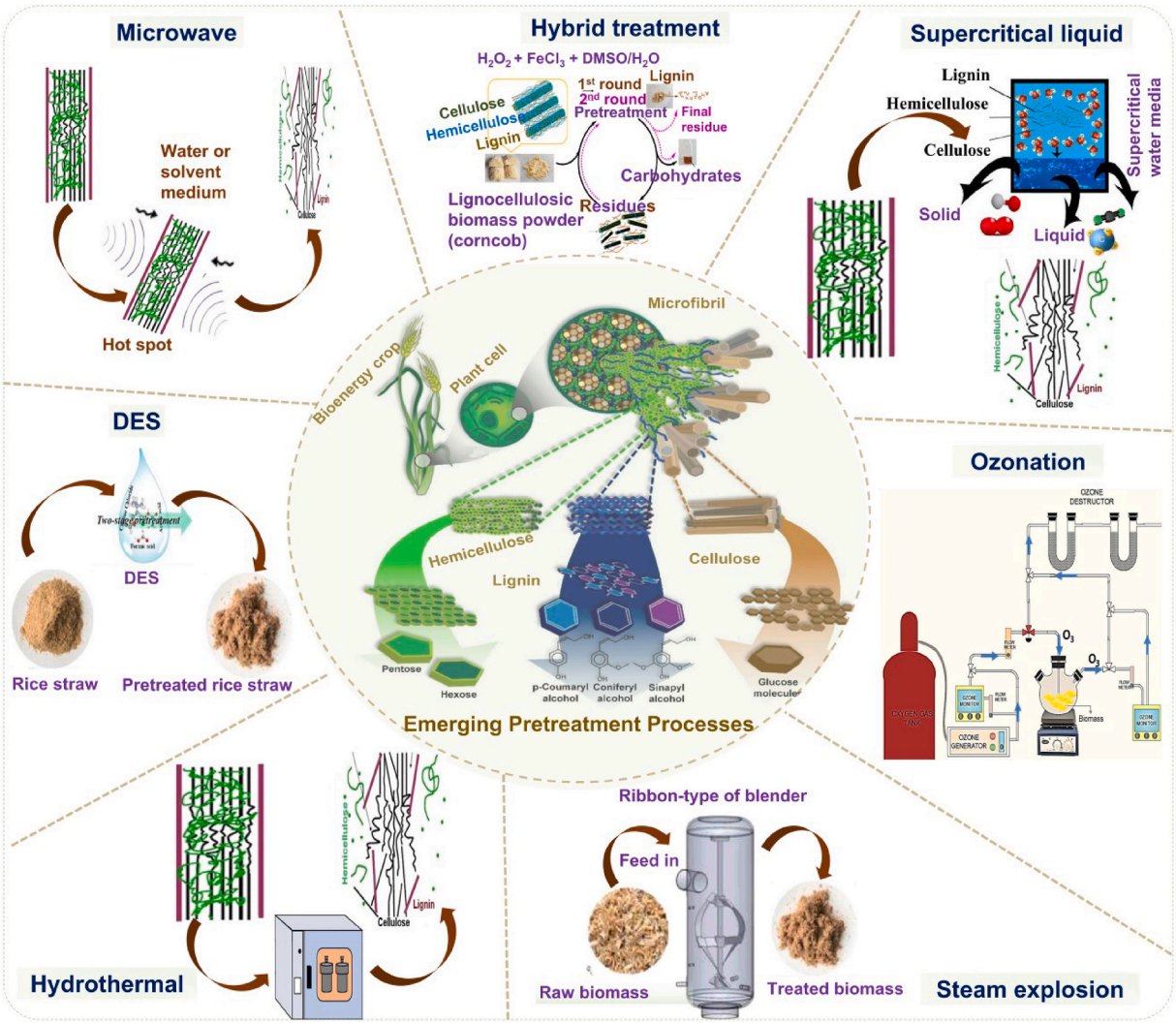


**Fig. 2.** Emerging physicochemical and chemical technologies used for the pretreatment of lignocellulosic biomass [adapted from Aguilar-Reynosa et al. (2017) with permission from Elsevier, License No. 5181230680094; Omar and Amin (2016) with permission from Elsevier, License No. 5181230300318; Tian et al. (2020) with permission from Elsevier, License No. 5181230946260; Xing et al. (2018) with permission from Elsevier, License No. 5181221467973; Liao et al. (2016) with permission from Elsevier, License No. 5181651314593; Hernández-Beltrán et al. (2019); Yu et al. (2018); Salimi et al. (2016)].

5-hydroxymethyl furfural (5-HMF)] under harsh pretreatment conditions (such as 60–75 min treatment with 4% HCl or 3.5% $H_2SO_4$ at 120–210 °C) (Barakat et al., 2012; Panakkal et al., 2022). By-products generated during LCB pretreatment negatively affect the fermentative efficiency by altering the metabolic pathways and dynamics of the microbial community involved in AD or fermentation. Weak acids (acetic, formic, and levulinic acids), generated during the further breakdown of 5-HMF and furfural, can diffuse into microbial cells through lipoprotein of the plasma membrane and cause cell lysis due to acidification of cytosol (Mirmohamadsadeghi et al., 2021). Fermentation medium containing 5-HMF concentration <3 g/L does not show any toxic effect on *C. saccharoperbutylacetonicum* during ABE fermentation. In contrast, lignin-derived phenolic compounds inhibit the growth of microbial cells even at low concentrations (<1 g/L) (Wang et al., 2019). These inhibitory by-products can penetrate the microbial cells by a disintegrating cell membrane, limits cell growth by lag phase elongation, inhibit glycolytic enzymes, and damage DNA depending upon their molecular structure, weight, and hydrophobicity (Devi et al., 2021). The phenolic compounds, 5-HMF, and furfural had more significant inhibitory effects on the microbes involved in dark fermentation than AD (Monlau et al., 2014).

The growth of specific beneficial species (*Clostridium beijerinckii*, *Sporolactobacillus putidus*, *Clostridium acetobutylicum*, *Clostridium cellulosi*) in the microbial consortium during dark fermentation may be suppressed by phenolic and furanic compounds, resulting in an overall low $H_2$ production (Basak et al., 2020a; Quéméneur et al., 2012). However, certain microorganisms, such as *C. beijerinckii,* can tolerate lignocellulosic inhibitors (phenolic compounds) and enhance their population from 70.7% to 85.8% during $H_2$ production from LCB (Quéméneur et al., 2012). Additionally, yeast cells can also sustain at low concentrations (<1 g/L) of furfural and 5-HMF by metabolizing them into relatively less harmful compounds, such as furfuryl alcohol and 2,5-bis-hydroxymethylfuran, respectively (Jonsson et al., 2013). Furfural, vanillin, and phenolic compound degrading fungal species *Byssochlamys nivea* was identified after screening 44 fungal species for efficient detoxification of lignocellulosic inhibitors in less than 11 days (Zanellati et al., 2021).

A variety of physical (heating and vaporizing) (Larsson et al., 1999), chemical (NaOH, $Ca(OH)_2$, $NaBH_4$) (Monlau et al., 2015), adsorption (activated carbon) (Mikulski and Kłosowski, 2020), liquid-liquid extraction (ethyl acetate, trialkylamine) (Monlau et al., 2015), enzymatic (laccase, peroxidase), and genetically modified microbe-based (Kannisto et al., 2015; Singh et al., 2019) processes can be used to detoxify the slurries and hydrolysates after LCB pretreatment (Table 4).

**Table 2**
Critical comparison of various emerging physicochemical technologies for the pretreatment of lignocellulosic biomass [adapted from Haldar and Purkait (2021) with permission from Elsevier, License No. 5181210241494; Hassan et al. (2018) with permission from Elsevier, License No. 5182810299067; Yiin et al. (2021) with permission from Elsevier, License No. 5182801386825].

| Pretreatment methods and process conditions | Impact on the degradation of the lignocellulosic substrate | Advantages | Disadvantages | Impact on production | | Ref. |
|---|---|---|---|---|---|---|
| | | | | Bio-$CH_4$ or $H_2$ | bio-ethanol | |
| DESs (e.g., ChCl/Glycerol) temperature: 40–110 °C | - Disintegration of the recalcitrance structure<br>- Fractionation of hemicellulose and partial removal of lignin | - Green and cheap solvents<br>- Bio-degradable and bio-compatible solvents<br>- Easy to prepare<br>- Low energy requirement | - Low stability under high temperature<br>- High viscosity<br>- Hygroscopic properties | + | + | Zhang et al. (2016) |
| ILs (e.g., Imidazolium-based complexes), temperature <100 °C | - Partial dissolution of lignin and hemicellulose<br>- Enhanced porosity and reduced crystallinity of cellulose | - Tailor-made solvents<br>- Low vapor pressure<br>- Mild operating conditions<br>- Efficient lignin recovery<br>- Easy to recover and recycle<br>- Thermally stable | - High operating cost due to complex synthesis and purification<br>- Poor biodegradability<br>- Inhibitory effect of enzymatic processing<br>- Industrial competitiveness | + | – | Yoo et al. (2017) |
| Supercritical fluids (e.g., SC-$CO_2$), pressure: 7–25 MPa, temperature: 200 °C | - Enhanced bioaccessibility for enzymatic saccharification<br>- Reduced crystallinity of cellulose and removal of lignin | - Green solvent<br>- Minimal loss of sugars<br>- Non-toxic and non-flammable<br>- Easy to recover | - High utility and equipment cost<br>- High operating cost<br>- Not suitable for the lignocellulosic substrates with low moisture content | + | + | Daza Serna et al. (2016) |
| Hydrothermal, temperature: 150–300 °C, pressure: 0–6 MPa, time: 10–240 min | - Fractionation of biomass components<br>- Facilitation of enzymatic hydrolysis (131% increase in glucose yield) | - No use of corrosive chemicals<br>- Eco-friendly process<br>- Short processing time | - High temperature and pressure are required<br>- Generate inhibitory by-products | + | + | He et al. (2015) |
| Steam explosion, temperature: 184–235 °C, pressure: 1.1–3.1 MPa | - Softening of lignin<br>- Reduced particle size<br>- Disintegration of the complex structure of LCB<br>- High sugar recovery | - Low capital investment<br>- Moderate energy requirement<br>- Limited use of chemicals<br>- Eco-friendly process with reduced generation of waste | - Less effective in softwood<br>- Formation of inhibitors to bioconversion | + | + | Pielhop et al. (2016) |
| Ozonization ($O_3$ and ethanol), oxidizing potential (EO = +2.07 V), pH: 3, ozone dose: 8 mg/L | - Reaction with olefins to form the ring structure and subsequent breakdown in smaller compounds<br>- Enhance porosity and biodegradability<br>- Reduced lignin content | - No use of corrosive chemicals<br>- Operation at ambient temperature and pressure, reduction in lignin content, and the release of sugars<br>- Strong oxidant nature of $O_3$ is effective<br>- No generation of toxic by-products | - Needs high ozone dosages for high biomass loadings<br>- Economically unfeasible at industrial scale<br>- Needs high energy<br>- $O_3$ is highly reactive, corrosive, and flammable | + | + | Mulakhudair et al. (2017) |
| MWI (needs $H_2O_2$, $NaClO_2$) | - Decreased crystallinity of cellulose<br>- Removal of lignin and degradation of hemicellulose | - Short processing time<br>- Easy to handle<br>- Quick heat transfer in biomass<br>- Environmentally benign process<br>- Selective, low residence time<br>- Low/no inhibitors generated at low temperature and pressure | - Microwave power is poorly distributed<br>- Uneven biomass heating<br>- Inhibitors are generated in severe conditions (high temperature and pressure)<br>- High capital and operating costs | + | + | Baruah et al. (2018) |
| Ultrasound | - Deconstruction of the biomass for enhanced enzymatic saccharification<br>- Reduced lignin content | - Highly efficient in sugar production<br>- Short pretreatment time<br>- Enhanced distribution of biomass | - Need high energy input<br>- Less feasible in large-scale application | + | + | Luo et al. (2014) |
| $CO_2$ laser pretreatment, solid to liquid ratio: 21.3 mL/g, power: 264.3 W, time: 67 min | - Increased surface area of biomass<br>- Decreased crystallinity of cellulose<br>- Enhanced enzymatic saccharification yield | - No strong and toxic chemicals are used<br>- Short processing time<br>- Low/no lignocellulosic inhibitors are generated | - High energy-demanding process<br>- Consumes power during $CO_2$ laser generation<br>- High capital and operating cost<br>- Needs renewable energy sources to make the process sustainable | + | + | Tian et al. (2011) |
| Plasma pretreatment, $N_2$/air (wet and dry), argon, and ozone gases which are ionized under high-voltage in-between two electrodes | - Destruction of chemical bonds or linkages within the complex structure<br>- Enhanced delignification<br>- Reduced cellulose crystallinity | - No need for strong chemicals<br>- Low/no conversion of lignin and/or sugars into inhibitors<br>- Short processing time<br>- Renewable energy sources can increase sustainability | - Requires high energy source<br>- Needs further investigation to know the treatment mechanism involved between the plasma-lignocellulose interaction<br>- High equipment cost | +<br><br><br><br>+ | +<br><br><br><br>+ | Gao et al. (2014) |

(*continued on next page*)

**Table 2** (*continued*)

| Pretreatment methods and process conditions | Impact on the degradation of the lignocellulosic substrate | Advantages | Disadvantages | Impact on production | | Ref. |
|---|---|---|---|---|---|---|
| | | | | Bio-$CH_4$ or $H_2$ | bio-ethanol | |
| PEF, Voltage pulses: 0.1–80 kV/cm; time: $10^{-2}$ s | - Disruption of biological membrane<br>- Reduced crystallinity of cellulose | - Non-thermal and low energy requirement<br>- No strong chemicals are used<br>- Short processing time | - Unsuitable for solid biomass<br>- Operational and safety issues<br>- Non-uniform pretreatment effect | | | Kovacic et al. (2021) |

'+/−' signs indicate a positive and negative impact on production.

**Table 3**
Effects of integrated physicochemical pretreatment methods on the biodegradability of lignocellulosic biomass [adapted from Haldar and Purkait (2021) with permission from Elsevier, License No. 5181220302245; Ong and Wu (2020) with permission from Elsevier, License No. 5181220518849].

| Integrated pretreatment methods | Lignocellulosic substrates | Operating conditions | Key findings | Ref. |
|---|---|---|---|---|
| Ultrasound and dilute acid | *Triarrhena lutarioparia* | Acid (HCl), 10% (w/v); Solid loadings, 10% (w/v); temperature, 120 °C; operating time, 1 h; ultrasonication for 30 min at 200 W and 20 kHz | Lignin content reduced to 17.6%; Reducing sugars increased from 79.4% to 111.2% | Tao et al. (2017) |
| Ultrasound and alkaline | Rice straw | Biomass loading, 20% (w/v); NaOH, 1% (w/w); ultrasonication for 1 h at 300 W and 22 kHz | Lignin content reduced to 4.6% | Wu et al. (2017) |
| Ultrasound and alkaline $H_2O_2$ | Sunn hemp | Biomass loading, 40% (w/v); $H_2O_2$, 2%; ultrasonication for 1 h at 130 W and 50 Hz at 50 °C | Lignin content reduced to 4.96% and pretreatment time reduced to 80% | Baksi et al. (2019) |
| Ultrasonic and IL | Rice straw | Biomass loading, 20% (w/v); time, 15 min; ultrasonication for 1 h at 70 °C | Increase in delignification up to 19% and total reducing recovery up to 29% | Han et al. (2018) |
| Ultrasound and DES | Oil palm frond | Biomass loading, 10% (w/v); ChCl: urea (1:2); ultrasonication for 30 min at 70% amplitude | Lignin removal up to 36.4% and xylose recovery by 58% | Ong et al. (2019) |
| Hybrid organic solvent and steam explosion | Spruce biomass | Biomass loading, 1% (w/w) sulphuric acid; ethanol, 50% (w/w); temperature, 200 °C; 30 min | Delignification up to 79.4%, enhanced cellulose content to 63.3%, saccharification yields 61% (w/w) | Matsakas et al. (2019) |
| Hybrid pretreatment | Corncob | Biomass loading, 2% (w/v) hydrothermal temperature, 130 °C, 1.5 bar pressure and treatment time for 0.5–4 h in modified Fenton's solution (0.007 mM $FeCl_3$, 0.3 mM $H_2O_2$ and 2 mL 1:6 ratio of DMSO: $H_2O$ solvent) | Delignification up to 87%, recovery of 94% of total carbohydrates | Yu et al. (2018) |
| Microwave and dilute acid | Stillage of wheat and rye | Biomass loading, 5% (w/v) in sulphuric acid (0.2 M); microwave at 0.372 MPa pressure for 15 min residence time at 300 W | Allowed cellulose hydrolysis yield up to 75% in pretreated stillages | Mikulski and Kłosowski (2020) |
| Microwave and alkali salt | Corn cob | Biomass loading, 10% (w/v); alkali NaOH (1.5 M) + different salts like $Na_3PO_412H_2O$, $Na_2CO_3$, and $CH_3COONa$; microwave at 700 W for 6 min | Sugar yield enhanced up to 7-fold with the highest saccharification of 0.8 g/g of reducing sugars using 11.6% $Na_3PO_412H_2O$ salt | (Sewsynker-Sukai and Gueguim Kana, 2018) |
| Microwave and hydrothermal method | Brewer's spent grain | Biomass loadings, 10% (w/v) in hydrothermal; temperature, 150–220 °C; residence time, 2–10 min; microwave at 50 Hz and 1000 W | No strong chemical used, 82% of fermentable sugars achieved at optimum condition, i.e., 193 °C for 5 min, 60 kg of ABE fermentation per ton of biomass | López-Linares et al. (2019) |
| DES and hydrothermal in acidic condition | Switch grass | Biomass loading, 20% (w/v); hydrothermal temperature, 130 °C; time, 30 min; ChCl: ethylene glycol (1:2) with 25% w/v; solid loading at temperature 130 °C for 30 min | Cellulose enriched to 72.6%, enzymatic saccharification yielded 241 g/L reducing sugars, i.e., 86% of glucose | Chen et al. (2018) |
| DES and microwave | Switch grass, corn stover, miscanthus | Biomass loading, 10% (w/v); microwave at 800 W for 45 s; ChCl: lactic acid, (1:2) | Delignification up to 85–87%, cellulose enrichment up to 65–67% | Chen and Wan (2018) |

However, most of these processes have limitations, such as the need for chemical supplements, heat, loss of sugars, additional pretreatment steps, and longer processing time (Basak et al., 2020a). Detoxication *via* adsorption successfully reduces the toxic stress due to the high sorption capacity of lignocellulosic inhibitors (furfural and 5-HMF) present in the hydrolysate without disturbing the sugar concentration. For example, Monlau et al. (2015) successfully removed 5-HMF (94%) and furfural (99%) using a pyrochar (4%, w/v) for 24 h in a synthetic medium. Similarly, coconut shell activated carbon (2%, w/v) efficiently adsorbed phenolic compounds and furaldehyde from hydrothermally pretreated sugarcane bagasse hydrolysate, without affecting sugars. The detoxified hydrolysate produced 30% more glucose during saccharification, while yielding 14-fold enhanced alcoholic fermentation (Freitas et al., 2019). Adoption of an appropriate LCB pretreatment method, under optimized conditions with low or no generation of toxic compounds and without compromising the yield of fermentable sugars, is more effective for obtaining high yield and purity and the valuable final product (bio-methane/$H_2$/ethanol/butanol) than subsequent detoxification steps (Mikulski and Kłosowski, 2020).

### 3.2. Biological pretreatment

In recent years, biological pretreatment using lignocellulolytic bacteria and fungi (brown, white, and soft rot) has emerged as an alternative, efficient, eco-friendly, and cost-effective approach for the pretreatment and depolymerization of lignocellulose without generating inhibitory byproducts (Mustafa et al., 2016) (Table 5). The efficacy of a lignocellulose-degrading microorganism is not an absolute value and varies mainly depending on the type as well as the structural and chemical composition of the LCB. The appropriate selection of microorganisms based on the lignocellulosic composition of biomass can render the process efficient, time-saving, and reduce substrate loss.

**Table 4**
Critical comparison of some effective approaches to detoxify the lignocellulosic inhibitors from hydrolysates.

| Biomass and pretreatment conditions | Hydrolysate composition | Detoxification process | Inhibitors removal | Pros and cons | Biofuel production | References |
|---|---|---|---|---|---|---|
| Switchgrass (*P. virgatumis*), 3 g/L acetic acid at temperature of 170 °C for 20 min | - Carbohydrates (5.8 g/L; xylose, 10.4 g/L, total carbohydrate, 20 g/L)<br>- Inhibitory products (acetic acid, 4.9 g/L; 5-HMF, 0.5 g/L; furfural, 0.8 g/L; phenolics, 6.3 g/L) | - Adsorption by activated carbon<br>- Adsorbent loaded 5% (w/v), agitated at 150 rpm at 60 °C for 6 h | - 50–60% removal of furfural and 5-HMF<br>- 50% removal of phenolics | Pros<br>- Cost-effective process<br>- Easy to handle<br>- Easy to regenerate<br>- Detoxify by physical adsorption on activated carbon<br>Cons<br>- Adsorption of carbohydrates<br>- Total carbohydrate reduced by 11% | - Simultaneous saccharification and fermentation<br>- ABE fermentation using *C. saccharoperbutylacetonicum* N1-4<br>- 8.6 g/L butanol (0.16 g/g yield) | Wang et al. (2019) |
| Olive tree clipping, 75 mM oxalic acid pretreatment at 150 °C | - Carbohydrates (glucose, 16.6 g/L; xylose, 15.1 g/L; arabinose, 2.8 g/L; 2.87 g/L)<br>- Inhibitory products (acetic acid; phenolic compounds, 3.18 g/L; furans, 0.96 g/L) | - Sodium borohydride as a reducing agent<br>- Used 0.03 mol/L for 30 min at 6 pH | - 94–98% reduction in furan concentration<br>- 40% reduction in phenolics content | Pros<br>- Mild reaction conditions<br>- Reduce phenolic and furanic content<br>- Positive effect on fermentation<br>Cons<br>- Furfural reduced to furfuryl alcohol causes toxicity to yeast cells<br>- 1.3 and 4% loss of glucose and xylose, respectively | - Fermentation by *Pichia stipitis* CBS 6054<br>- 3.8 g/L ethanol (27% yield) at 63 h | Peinado et al. (2019) |
| Cassava stem, 20% (w/v) loading, Two-step acid pretreated (72%, w/w $H_2SO_4$ added 1:1.25 ratio) biomass stirred for 1 h and autoclaved at 111 °C for 1 h | - Carbohydrates (glucose, 95 g/L)<br>Inhibitors (furfural, 0.95 g/L | - Liming by $CaCO_3$ to adjust pH up to 11<br>- Adsorption by 10% (w/v) activated carbon by agitating 1 h | - Furfural removal | Pros<br>- Easy handling<br>- Completely removed furfural from hydrolysate<br>- Activated carbon can be reused<br>Cons<br>- Solid waste (gypsum) formed<br>- Frequent pH change<br>- Glucose concentration reduced up to 27% | - *S. cerevisiae* strain IAM4178<br>- intermittent yeast inoculation produced 37.5 g/L ethanol | Tanaka et al. (2019) |
| Hemicellulosic hydrolysate (sugarcane bagasse), Pretreated with 0.5% (v/v) $H_2SO_4$ at 140 °C for 15 min | - Carbohydrate (glucose, 15.4 g/L; xylose, 200.5 g/L; arabinose, 16.1 g/L; cellobiose, 4.6 g/L)<br>- Inhibitory products (acetic acid, 28 g/L; formic acid, 0.38 g/L; phenolics, 12.4 g/L; 5-HMF, 0.15 g/L; furfural, 0.12 g/L | - Vacuum evaporation followed by liquid-liquid extraction by methyl-isobutyl-ketone | - 85.4% acetic acid removal<br>- 69% phenolics removal<br>- 100% removal of formic acid, 5-HMF, and furfural | Pros<br>- Less sugar loss (~5% glucose and 1% xylose)<br>- 98.8% reducing sugars were consumed by yeast after detoxification<br>- 4.4-times increase in ethanol yield after detoxification<br>Cons<br>- High operating cost<br>- high consumption of solvent<br>- Additional solvent recovery step is required | - Ethanol yield and productivity by *S. stipitis* 71.8% and 0.38 g/L/h, respectively and by *S. passalidarum*, 82.2% and 0.91 g/L/h, respectively in 48 h | Roque et al. (2019) |
| Sugarcane bagasse, 15% (w/v) biomass loading, Hydrothermal pretreatment at 195 °C, 200 rpm for 10 min | - Carbohydrates (glucose, 48.02 g/L; xylose, 18 g/L)<br>- Inhibitors (phenolics, 2.68 g/L) | - Washing of hydrolysate<br>- Addition of soybean protein during enzymatic saccharification | - 72% reduction in phenolics | Pros<br>- Soybean proteins prevent unproductive adsorption of cellulases on lignin<br>- 18% enhanced saccharification after addition of soybean proteins<br>Cons | - 75% theoretical yield of ethanol | Pinto et al. (2021) |

(*continued on next page*)

**Table 4** (*continued*)

| Biomass and pretreatment conditions | Hydrolysate composition | Detoxification process | Inhibitors removal | Pros and cons | Biofuel production | References |
|---|---|---|---|---|---|---|
| | | | | - Addition of soybean protein delay the hydrolysis process in initial 12 h | | |
| Lodgepole pine (*P. contorta*), woody biomass, Acid (1%, w/w $H_2SO_4$) pretreated at 180 °C for 40 min followed by steam pretreatment at 200 °C for 5 min | - Carbohydrates (glucose, 55.4%; xylose, 1.4%; mannose, 2%)<br>- Inhibitory products (acid-insoluble lignin, 44.6%; acid-soluble lignin, 1.1%) | - Addition of carbocation scavengers (hydroxybenzoic acid, vanillic acid, and syringic acid) dissolved in acetone (5%, w/w)<br>- Scavengers agitated with biomass for 2 h | - Addition of carbocation scavengers efficiently mitigate the toxic effect of lignin-derived phenolics during saccharification | Pros<br>- No need for additional detoxification step<br>- Cellulose hydrolysis increased up to 50% by adding syringic acid<br>Cons<br>- Steam pretreatment is required after the addition of scavenger to the acid pretreated biomass | [a]N.R. | Zhai et al. (2018) |
| Wheat straw biomass (10%, w/v), Acid (0.5%, w/v $H_2SO_4$) pretreated at 140 °C stirred at 300 rpm for 1h | - Carbohydrates (glucose, 50.7%; xylose, 6.9%)<br>- Inhibitory product (lignin, 39.6%) | - Addition of 1 mM $MgCl_2$ | - Reducing the negative charges over the surface of lignin<br>- Reduces the toxicity of lignin<br>- $Mg^{2+}$ block Ph-OH groups of lignin monomeric units | Pros<br>- Weaken the unproductive binding of cellulase to lignin<br>- Improved saccharification by 19.3% at low enzyme loading (5 FPU/g solid)<br>Cons<br>- Metal ions may interfere with the saccharification process<br>- Efficiency depends upon pH, operating time, and enzyme loading | N.R. | Akimkulova et al. (2016) |
| Sugarcane hydrolysate, Acid (1.25%, w/w $H_2SO_4$) pretreated biomass autoclaved at 121 °C for 2 h | - Carbohydrates (glucose, 7.74 g/L; xylose, 16.5 g/L)<br>- Inhibitory products (acetic acid, 3.3 g/L; 5-HMF, 1.03 g/L; furfural, 0.42 g/L) | - Biodetoxification using newly isolated strain, *Bordetella* sp. BTIITR from soil | - Degrade 100% furfural, 94% 5-HMF and 82% acetic acid in 16 h | Pros<br>- Cost-effective, efficient, and eco-friendly process<br>- Lessen to inhibitors for smooth saccharification and fermentation process<br>Cons<br>- Biodetoxification is a slow process | N.R. | Singh et al. (2017a) |
| Wood chip (poplar), Steam pretreatment at 170 °C for 2 h, liquid hydrolysate is treated with 4% (w/v) $H_2SO_4$ | - Carbohydrates (glucose, 3.5 g/L; xylose, 81.7 g/L; arabinose, 4.1 g/L)<br>- Inhibitory products (acid-soluble lignin, 34 g/L; formic acid 4.3 g/L; acetic acid, 21 g/L; benzoic acid, 0.022 g/L) | - Ion-exchange resin IRA-400 ($OH^-$) | - 96.7% of acid-soluble lignin was removed<br>- Significant removal of acetic acid (43.9%), benzoic acid (95%) | Pros<br>- IERs are chemically and mechanically stable<br>- Efficiently removed phenolic compounds<br>- Regeneration and reuse make the IER cost-effective<br>Cons<br>- Need acid/base for regeneration<br>- Generates wastewater during washing and regeneration<br>- 21% sugar (xylose) lose | - 95.3% of total sugars are utilized by *S. cerevisiae* in 72 h - Ethanol concentration increased to 41.5 g/L with a yield of 89.6% | Yu and Christopher (2017) |

[a] Not reported.

**Table 5**
A comparison of various biological pretreatment strategies for lignocellulosic biomass.

| Pretreatment microorganisms/Enzyme preparations | Lignocellulosic feedstock | Culture technique | Pretreatment incubation condition | Impact of pretreatment on biomass | Impact of pretreatment on anaerobic bioprocess | Ref. |
|---|---|---|---|---|---|---|
| Mixed microbial consortium | Sawdust waste | Submerged | $X_0/S_0 = 12$ mg/g; incubated at 30 °C for 10 d | Reduction in TS (83.4), VS (56.9%), cellulose (37.5%), hemicellulose (39.6%), and lignin (56.7%) | 25.6% higher biogas yield after 28 d of AD | Ali et al. (2017) |
| *Pleurotus pulmonarium* MTCC 1805 | Sugarcane top | Solid-state | Incubated at 28 °C for 21 d | Reduction in lignin (60.4%), hemicellulose (24.3%), and cellulose (5%); TS recovery was 81.7% | 37.7% and 54% higher yields of bio$H_2$ and bio$CH_4$, respectively | Kumari and Das (2016) |
| *Citrobacter werkmanii* VKVVG4 | Whole water hyacinth plant | Submerged | Incubated at 37 °C and stirred at 120 rpm for 4 d | Reduction in cellulose (30.8%) and hemicellulose (43%); overall solubilization of the biomass, 33.3% | 23% increase in cumulative biogas production | Barua et al. (2018) |
| *Aspergillus fumigatus* NITDGPKA3 | Mixed fruit and vegetable wastes | Submerged | $X_0/S_0 = 2 \times 10^5$ spores/g; incubated at 30 °C and stirred at 180 rpm for 5 d | Soluble carbohydrate recovery was 362.84 mg/g; reduction in hemicellulose (62.7%), cellulose (53.6%), and lignin (8.8%) | 53% higher biohythane yield, which corresponds to 47% higher energy recovery | Basak et al. (2020b) |
| *Phlebia brevispora* NRRL-13108 | Corn Stover | Solid-state | Moisture content 84%, incubated at 28 °C for 42 d | Total sugar yield was 442 mg/g; reduction in hemicellulose (34.9%), cellulose (15.1%), and lignin (41.9%) | Bioethanol yield, 0.16 g/g pretreated biomass | Saha et al. (2017) |
| Mixed microbial consortium | Corn stover | Submerged | Incubated at 37 °C for 6 d | Degradation of cellulose (35.6%), hemicellulose (43.6%), and lignin (13.98%) | Increased bio$CH_4$ production by 62.8%; increased activity of *Methanosaeta* | Zhao et al. (2019) |
| Basidiomycete *Irpex lacteus* | Corn stover, barley straw, corncob, and wheat straw | Solid-state | Incubated at 30 °C for 21 d | Degradation of glucan, xylan, and lignin were 18, 6.3, and 16.7% for wheat straw; 4, 32.8, and 29.3% for corn stover; 17.1, 37.7, and 4.3%; for corncob and 19, 20, 6.4%, for barley straw | Bioethanol yield, 79–106 mg/g pretreated biomass | García-Torreiro et al. (2016) |
| *Gymnopus contrarius* J2 | Rice straw | Submerged | Moisture content 65%, incubated at 25 °C in a static condition for 21 d | Removal of lignin (41.9%), cellulose (22.9%), and hemicellulose (22.7%); decrease of cellulose crystallinity index by 46.2% | 74% increase in bio$H_2$ yield | Sheng et al. (2018) |
| *Pleurotus ostreatus* DSM 11191; *Trichoderma reesei* QM9414 | Rice straw | Solid-state | Moisture content 65, 75, and 85%; incubated at 28 °C for 30 d | Removal of TS (13.2%) and degradation of hemicellulose (23.7%), lignin (35.3%), and cellulose (13.1%) with *P. ostreatus* pretreatment; removal of TS (10.6%) and degradation of hemicellulose (23.3%), lignin (23.6%), and cellulose (20.1%) with *T. reesei* pretreatment | Higher methane yield of 120% and 78.3% when pretreated with *P. ostreatus* and *T. reesei*, respectively | Mustafa et al. (2016) |
| Mixed microbial consortium | Corn straw | Submerged | Incubated at 30 °C for 14 d | Degradation of hemicellulose (44.4%), cellulose (34.9%), and lignin (39.2%) | 131.6% higher methane production compared to the control | Li et al. (2020) |
| *Paecilomyces inflatus* ATCC 32919 | Surgical waste cotton and waste cardboard | Submerged | Incubated at 28–30 °C and 180 rpm for 3 d | Degradation of hemicellulose (76%) and lignin (25%); cellulose crystallinity index decreased by 20.1% | Fermentation with *Saccharomyces cerevisiae* RW143 yielded 51.2 g/L ethanol with 58.4% cellulose conversion | Ramamoorthy et al. (2020) |
| *Flammulina velutipes* | *Agropyron elongatum* (Tall wheatgrass) | Submerged | Moisture content 45, 60, 75%; incubation at 28 °C for 28 d | Maximum removal of hemicellulose (29.1%), lignin (35.4%), and cellulose (20.5%) | 120% increased biogas production with 134% higher methane yield | Lalak et al. (2016) |
| Celustar XL (Endoglucanase, xylanase), Agropect pomace (pectinase) | Sugar beet pulp silage and vinasse | Submerged enzymatic hydrolysis | Incubated at 50 °C for 7 d | Release of reducing sugar (87.3–92%) | 27.9% enhancement in biogas yield | Ziemiński and Kowalska-Wentel (2015) |
| | Corn stover | Submerged | | | | |

(*continued on next page*)

**Table 5** (*continued*)

| Pretreatment microorganisms/Enzyme preparations | Lignocellulosic feedstock | Culture technique | Pretreatment incubation condition | Impact of pretreatment on biomass | Impact of pretreatment on anaerobic bioprocess | Ref. |
|---|---|---|---|---|---|---|
| A mixture of laccase, manganese peroxidase, and versatile peroxidase | | | Incubated at 30 °C under continuous shaking for up to 24 h | Degradation of lignin fraction and release of lignin-derived phenolic compounds | 17–25% increase in biomethane yields | Schroyen et al. (2014) |
| Co-immobilization of laccase, cellulase, and *β*-glucosidase | Giant reed (*Arundo donax*), Energy cane (*Saccharum arundinaceum*), Cattail (*Typha angustifolia*), and Morning glory (*Ipomoea carnea*) | Submerged | Incubated at 30 °C and stirred at 150 rpm for 60 h | Reduction in cellulose crystallinity index by 5.7–18.4%; maximum TRS ranged from 158 to 205 mg/g of different pretreated biomass | Increased ethanol yield ranged from 52.1 to 63.4% for the fermentation of varying biomass with *S. cerevisiae* | Kirupa Sankar et al. (2018) |
| Commercial enzymes Celluclast 1.5L (Cellulase) and Novozyme 188 (Cellolbiase) | Energy crops *Miscanthus giganteus* and *Sida hermaphrodita* | Submerged enzymatic hydrolysis | Incubated at 50 °C under shaking for 24 h | Degradation of hemicellulose, cellulose, and lignin were 94.9, 74.5, and 86.6%, respectively for *Miscanthus giganteus*; and 86.9, 76.7, and 35.4%, respectively for *Sida hermaphrodita* | Higher cumulative biogas production for *M. giganteus* (230.5 $dm^3$/kg TS) and *S. hermaphrodita* (223.7 $dm^3$/kg TS) | Michalska et al. (2015) |
| Ultraflo® L (Novozymes, Denmark), a cocktail of enzymes, including endo-1,3 (4)-*β*-glucanase, collateral xylanase, cellobiase, and cellulase. | Corncob | Solid-state | Incubated at 50 mM Na-phosphate buffer with solid: liquid 1:10 (w/v) at pH 6.0 for 3 h and stirred at 150 rpm at 40 °C | Reduction in cellulose (43.3–57.5%) and neutral detergent soluble compounds (41.7–43.5%) | Up to 22.3% increase in biogas production | Pérez-Rodríguez et al. (2017) |

### 3.2.1. LCB pretreatment by fungi

Fungi have been extensively employed for hydrolyzing LCB with high lignin content. Several members of Basidiomycota, such as white rot fungi, are superior lignin degraders owing to the presence of various lignases, including lignin peroxidases, laccases, and manganese peroxidases (Zhao et al., 2019). In the last decade, several soft and brown-rot fungi, such as *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum, Trichoderma ressei, Gloeophyllum trabeum, Coniophora puteana,* and *Postia placenta* have been used for hydrolyzing various LCBs to enhance the performance of anaerobic bioprocesses and improve the biofuel yield (Table 5). While soft and brown rot fungi can degrade LCB *via* their potent cellulolytic enzymes, they can only partially modify lignin to access the polysaccharidic portion (Abdel-Hamid et al., 2013).

White-rot fungi possess an extensive extracellular enzymatic machinery that produces hydrolases that catalyze cellulose and hemicellulose degradation. In addition, its ligninolytic system mediates lignin degradation, thereby increasing the overall bioavailability of the biomass for improved anaerobic bioprocessing (Table 5). Although white-rot fungi cannot utilize lignin as a sole carbon source, they are capable of entirely mineralizing lignin present in LCB while consuming cellulose and hemicellulose as an energy and carbon source (ten Have and Teunissen, 2001). However, the consequent loss of carbohydrates could be minimized *via* selective degradation of target substrates, such as hemicellulose and lignin, by a few species of white-rot fungi, leaving the cellulose fraction intact (Kainthola et al., 2021). For example, *Pleurotus ostreatus* is the most efficient fungi in selectively degrading lignin, with a high selectivity value (lignin/cellulose loss), compared to *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, and *Trametes versicolor*. However, these fungi also face a formidable challenge in lignin degradation because of their large and stereo-irregular structures. Despite the high lignocellulolytic activity of fungi in general, depending on the fungal strain and biomass, fungal pretreatment may lead to significant loss of soluble carbohydrates and an extensive pretreatment period (Mustafa et al., 2016). To overcome these issues, fungal strains with high lignin selectivity values and optimized process parameters should be considered for the pretreatment or delignification of LCB.

### 3.2.2. LCB pretreatment by bacteria

Employing lignocellulolytic bacteria can be a promising alternative to fungi for LCB pretreatment, as faster bacterial growth and metabolic rate allow a shorter pretreatment time. Several bacterial species belonging to Actinobacteria, α-Proteobacteria, and γ-Proteobacteria can effectively degrade lignin under aerobic conditions (Bugg et al., 2011). Compared to white-rot fungi, bacteria are not regarded as potent degraders of lignin (Baba et al., 2017; Rashid et al., 2017). Although bacteria cannot degrade native lignin anaerobically, they can degrade lignin fragments (Khan and Ahring, 2019). Several aerobic bacteria, such as *Pseudomonas* sp., *Bacillus* sp., *Sphingobium* sp., and *Rhodococcus* sp., can degrade lignin *via* oxidative reactions catalyzed by lignin-degrading extracellular oxidative enzymes, such as heme peroxidases (Ruiz-Dueñas and Martínez, 2009). Shi et al. (2017) described the degradation and bioconversion of Kraft lignin as the sole carbon source by the *β*-proteobacterium strain *Cupriavidus basilensis* B-8, which resulted in 41.7% lignin removal in 7 d.

Microaerobic pretreatment of LCB with a hydrolytic bacterial inoculum under thermophilic conditions can accelerate lignocellulose hydrolysis, reduce cellulose crystallinity, and improve biogas productivity in AD (Fu et al., 2015). Jaron et al. (2021) pre-digested the pasteurized organic wastes (e.g., waste activated sludge and dairy manure) using a hyperthermophilic bacterium, *Caldicellulosiruptor bescii,* at 75 °C and pH of 7–8, followed by AD at mesophilic condition (37–41 °C) to get the 2-fold increase in biogas production during the lab- and pilot-scale tests in comparison to control one. They suggested that 75–85% conversion of volatile solids into biogas containing 75% methane could be achieved commercially using *C. bescii* pre-digested organic waste streams.

Cellulolytic bacteria, such as *Clostridium* sp., *Cellulomonas* sp., *Citrobacter* sp., *Bacillus* sp., and *Streptomyces* sp., have been increasingly used in the pretreatment of lignocellulose during biogas production (Table 5) (Sharma et al., 2019). Some rumen and soil cellulolytic bacteria, such as *Ruminococcus* sp., *C. thermocellum*, *C. cellulolyticum*, and *C. cellulovorans* produce large multi-enzyme complexes containing cellulases, hemicellulases, and other carbohydrate-active enzymes called cellulosomes (Artzi et al., 2017) which can efficiently hydrolyze cellulose and hemicellulose into fermentable soluble carbohydrates during pretreatment.

### 3.2.3. LCB pretreatment by microbial consortia

Synergistic actions of lignocellulolytic enzymes produced by different microorganisms in a mixed culture or consortium can enhance lignocellulose biodegradation and solubilization during pretreatment. The use of microbial consortia in lignocellulose pretreatment has several advantages over the use of pure microbial culture: the higher hydrolytic capacity of the consortium results in maximum pretreatment efficiency in a relatively shorter time; no requirement to maintain optimum conditions for the pure culture; mixed consortia do not require aseptic conditions, thus minimizing the pretreatment cost (Wen et al., 2015). Therefore, the use of microbial consortia for LCB pretreatment has emerged as a promising technological breakthrough. Several studies have successfully developed microbial consortia specialized for lignocellulose degradation and pretreatment (Tabatabaei et al., 2020a). Most of these studies have followed the principle of enriching the lignocellulolytic microorganisms from specific sources, such as cattle manure and rumen fluid (Baba et al., 2017; Takizawa et al., 2018), compost of straw and/or other lignocellulosic materials, forest soil, rotten wood (Zhang et al., 2021), termite gut (Lazuka et al., 2018). In designing the microbial consortia, the primary aim of enrichment is to increase the number of specific communities of microorganisms that produce and secrete potent lignocellulolytic enzyme systems, such as carbohydrate-active enzymes (CAZymes) cellulosomes and laccase capable of degrading lignocellulose effectively. For example, a lignocellulolytic microbial consortium enriched from wheat straw compost revealed the concoction of laccase producing white and brown rot fungi with cellulolytic members belonging to Firmicutes, Bacteroides, and Proteobacteria (Zhong et al., 2016). Baba et al. (2017) used the cattle rumen microflora to pretreat rapeseed biomass. They found that *Ruminococcus* sp. dominated the microflora, which could significantly degrade the lignocellulosic biomass. Dumond et al. (2021) applied termite gut microbial consortium including *Microcerotermes parvus*, *Termes hospes*, *Nasutitermes ephratae*, and an unidentified species closely related to *N. lujae*, which could degrade lignin (up to 37%), hemicellulose (51%), and cellulose (41%) effectively. However, in-depth research on this aspect is still limited, possibly due to difficulty in obtaining and culturing termite gut microorganisms (Zhao et al., 2022).

Microbial consortia, including bacteria (Maki et al., 2014), fungi (Kalyani et al., 2013), and mixed bacterial and fungal (Fang et al., 2018), have been used in lignocellulose pretreatment over the last decade to increase bioconversion efficiencies. Fang et al. (2018) employed a bacteria-fungal consortium for the selective degradation of lignin from tree trimmings, which resulted in high delignification after 16 d pretreatment, with a selectivity value of 2.78. Wen et al. (2015) evaluated three microbial consortia for pretreating Napier grass, resulting in 1.32- to 1.49-fold higher methane production in AD than the untreated control. A novel lignocellulose-degrading microbial consortium was isolated from rotten sawdust, significantly reduced cellulose (37.5%), hemicelluloses (39.6%), and lignin (56.7%) content after 10 d pretreatment, resulting in a 25.6% higher methane yield in AD (Ali et al., 2017). Despite these encouraging results, using microbial consortia in real-scale LCB pretreatment is associated with technological challenges, such as long-term maintenance of effective microorganisms in the consortium, need for optimal conditions for synergistic enzyme activities, and loss of soluble carbohydrates and other substrates due to the diversity of microorganisms present in the consortium (Zabed et al., 2019).

### 3.2.4. Enzymatic pretreatment of LCB

Enzymatic pretreatment of LCB employs purified, semi-purified, or crude enzymes (oxidative and hydrolytic), which are principally produced by bacteria and fungi (Table 5). Enzymatic pretreatment is gaining attention due to comparatively short reaction periods, low nutrient demand for enzymatic reactions, and more accessible process control. Moreover, most cellulolytic enzymes as free catalysts are less sensitive to lignocellulosic inhibitors compared to whole microbial cells as these enzymes vary in their affinity to the inhibitors (Li and Zheng, 2017). A microbial cell has many sites on different enzymes, proteins, and other macromolecules where these inhibitors can act upon and harm microbial cells (Basak et al., 2020a). Studies also indicated that some of the lignocellulosic inhibitors could even have a stimulating impact on the activity of certain lignocellulolytic enzymes. According to Tian et al. (2013b), the addition of lignocellulosic inhibitors, such as ferulic acid, *p*-coumaric acid, and salicylic acid, boosts the activity of cellulase against filter paper by 28.3, 15.1, and 10.1%, respectively. In enzymatic pretreatment, lignocellulolytic enzymes are also not affected by microbial metabolic products, such as organic acids and alcohols, because of the absence of microbial metabolism that produces these products. Using a single enzyme (e.g., cellulase) for pretreatment may result in inefficient saccharification of lignocellulose and a longer pretreatment time. However, physicochemical pretreatment followed by enzymatic saccharification has often been used for better soluble carbohydrate yields (Pérez-Rodríguez et al., 2017). Alkaline (NaOH)-pretreated biomass (*Miscanthus giganteus* and *Sida hermaphrodita*) followed by enzymatic hydrolysis using cellulase (Celluclast 1.5L) and cellobiase (Novozyme 188) resulted in higher saccharification with 20 g/L glucose, which subsequently yielded high methane in AD (Michalska et al., 2015).

Additionally, mixtures of several enzymes ensure synergistic action on different lignocellulosic biomass components, leading to enhanced hydrolysis and solubilization of biomass. For example, co-immobilized laccase, cellulase, and $\beta$-glucosidase used for LCB pretreatment decreased cellulose crystallinity and led to a high sugar yield of 205 mg/g (Kirupa Sankar et al., 2018). Similarly, crude enzyme cocktails extracted from fungal or bacterial cultures acclimatized to lignocellulose showed greater hydrolytic efficacy than pure enzymes. Copper-dependent enzymes, such as lytic polysaccharide monooxygenases (LPMOs), are designated as novel hydrolytic cocktails to depolymerize LCB. Sepulchro et al. (2021) isolated and characterized MtLPMO9A from *Thermothelomyces thermophilus* M77 which could be efficiently activated in the presence of light and chlorophyllin for photocatalysis of recalcitrant and crystalline cellulose by utilizing molecular oxygen or hydroperoxide as co-substrate. This indicates that the presence of a wide range of enzymes in the crude extracts, with ligninolytic and cellulolytic activities, can degrade cellulose fibers, reduce their crystallinity, and enhance saccharification (Asgher et al., 2013). Despite its advantages, enzymatic pretreatment has several drawbacks, such as a lower delignification rate than chemical methods (Asgher et al., 2013), longer pretreatment time, and high hydraulic retention time (Zabed et al., 2019). The overall performance of enzymatic pretreatment of lignocellulose is yet to be evaluated thoroughly. A techno-economic analysis should be performed compared to established physicochemical procedures for possible real-scale implementation.

## 4. Integration of multi-omics approaches in effective utilization of LCB

The multi-omics approach provides mechanistic insights into the intricate regulatory nexus that controls gene expression, alteration in protein structure, and composition of metabolites in an organism (de Figueiredo et al., 2021; Patil et al., 2020). Diverse meta-omics technologies, such as genomics, proteomics, transcriptomics, and metabolomics, have improved the accuracy of identifying various microbial populations, determining functional gene expression and protein interactions, and interpreting the metabolic pathways for an in-depth understanding of the LCB degradation (Aylward et al., 2012; Rosewarne et al., 2014). In addition, several meta-omics studies have revealed that members belonging to phyla Firmicutes and Bacteroidetes transcribe a diverse set of genes encoding cellulolytic glycosidic hydrolases and sugar transporters, which are ubiquitously involved in LCB degradation (Hassa et al., 2018). Thus, the combined information facilitates understanding metabolic pathway regulation and identifying

efficient organisms and key enzymes for the effective utilization of LCB.

### 4.1. Genomics

DNA sequencing using next-generation sequencing (NGS) and whole-genome assembly offers crucial information for identifying functional genes and mapping proteins and mRNA sequences (Gruninger et al., 2019). Metagenomics is a powerful tool for studying unculturable microorganisms present in environmental samples (Rane et al., 2022). It can be used to characterize microorganisms that grow on multiple substrates or under different environmental conditions (Mhuantong et al., 2015). Using the metagenomic approach, identifying and isolating microorganisms with beneficial traits, such as faster growth rate, higher substrate utilization rate, high productivity, and resistance to toxic metabolites may become possible. Notably, the metagenomic approach has been applied to environmental samples and allowed identification of functional gene loci, the discovery of diverse enzymes, including uncharacterized enzymes, and functional domains specialized for specific functions (Xia et al., 2016b). Functional metagenomics of the anaerobic consortium is used to predict polysaccharide utilization loci (PUL) (Tomazetto et al., 2020).

Metagenomic analysis of microbiota utilizing LCB can lead to strain isolation, identification, characterization of carbohydrate-degrading enzymes, and enrichment of potential bacterial strains to enhance the conversion of lignocellulosic substrate into biofuel (Fig. 3a) (Mhuantong et al., 2015). Moreover, 16S rRNA gene sequencing and metagenomics have helped to identify the dominant LCB-degrading species and their diversity (Wei et al., 2015). Operational taxonomic unit (OTU) analysis is used to determine the specific microbial strains or communities that are well adapted for degrading a particular lignocellulosic substrate (Campanaro et al., 2020). Moreover, sequence-based, and function-based metagenomics studies have filled the gap between the sequence and functions of the genome, thereby linking metabolic activity with the corresponding microbial species. Metagenomic studies have also focused on screening biomass-degrading enzymes in the CAZymes families to identify the efficient enzymes and analyze their functional diversity (Abot et al., 2016). Mhuantong et al. (2015) analyzed the metagenome *via* DNA sequencing of the fosmid library of the microbial community inhabiting the ecological niche at the bagasse collection sites of sugar mills. Potential biomass-degrading microbial communities and their lignocellulolytic enzymes were characterized through shotgun pyrosequencing of the library. Furthermore, various conserved genes responsible for LCB degradation were identified by comparing the microbial composition in different metagenomic data.

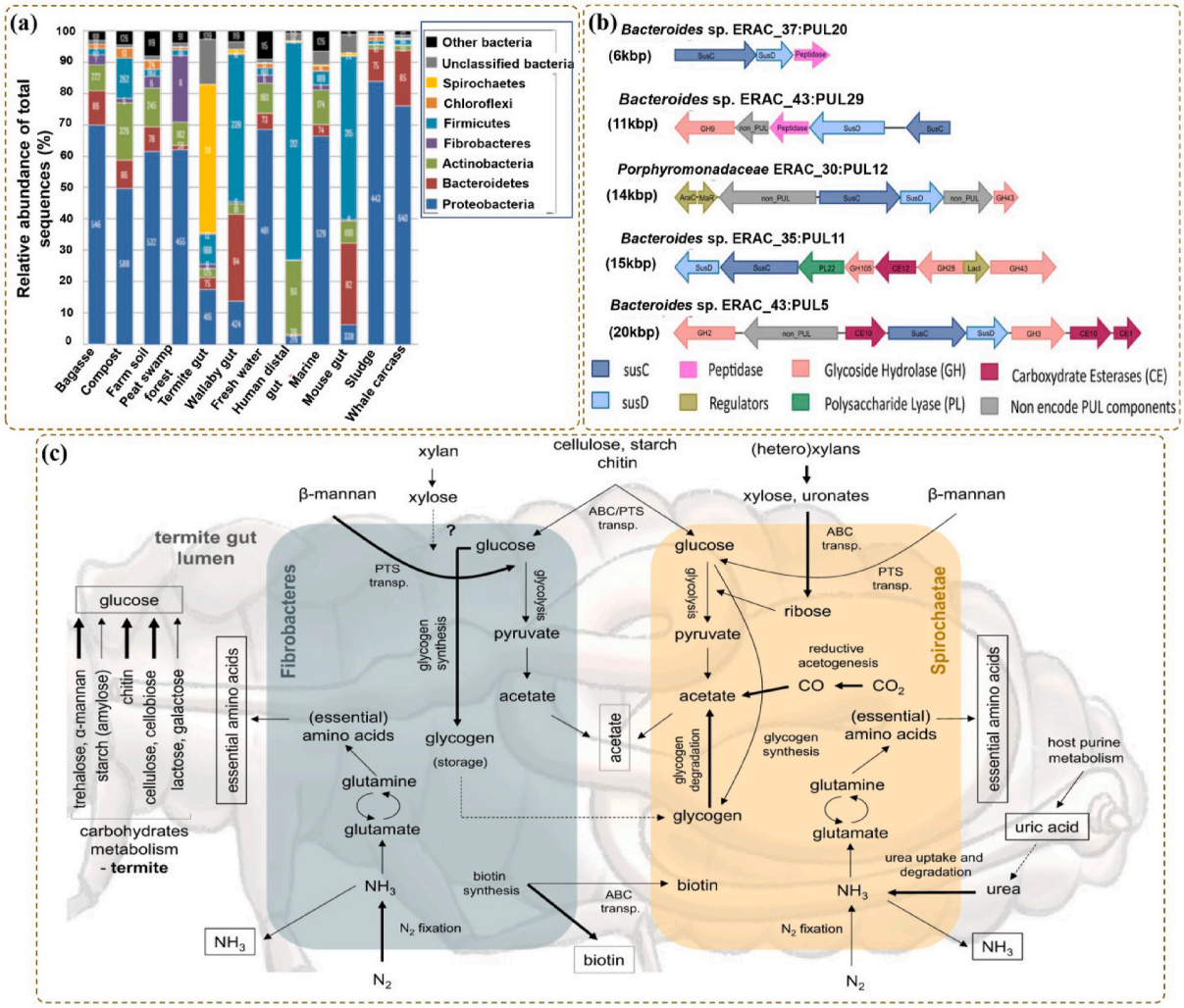


**Fig. 3.** Application of multi-omics approaches for the enhancing lignocellulosic biomass utilization; a) structural composition of lignocellulolytic microbial community based on 16S rRNA gene sequencing (Mhuantong et al., 2015), b) prediction of polysaccharide utilization loci (PUL) from the anaerobic consortium metagenome (Tomazetto et al., 2020), c) carbohydrate metabolites and predicted pathways in termite gut reconstructed using integrative omics data analysis (Calusinska et al., 2020).

### 4.2. Transcriptomics

RNA sequencing provides a blueprint of expressed genes and changes in gene expression patterns under a set of metabolic conditions. An in-depth study of the transcriptome will aid in determining the biological mechanisms used by bacterial species for degrading lignocellulose (Qi et al., 2011). Transcriptomic analysis of the termite gut and soil microbiota grown on different lignocellulose substrates has offered new insights into the microbiota response to environmental conditions at a functional level (Poretsky et al., 2009; Raychoudhury et al., 2013). Gene ontology analysis provides essential information regarding genes whose expression is necessary for degrading LCB and genes required to detoxify undesirable metabolites (Stewart et al., 2012). Multiple metagenomic and metatranscriptomic studies have identified approximately 132 families or subfamilies of CAZymes that actively participate in LCB utilization. Metatranscriptomic analysis of CAZymes of rumen microbes has confirmed the multi-fold enhancement in expression of genes (transcription) involved in carbohydrate metabolism (Fig. 3b) (Huttner et al., 2017; Qi et al., 2011).

Furthermore, the expression of members of CAZyme families such as GH5, GH6, GH9, and GH48 was upregulated in rumen anaerobes (Dai et al., 2015; Sollinger et al., 2018). The transcriptomic profile of *Laetiporus sulphureus* ATCC 52600 growing on sugarcane bagasse showed an upregulated mRNA expression of genes encoding redox enzymes along with those of cellulases and hemicellulases (de Figueiredo et al., 2021). An in-depth transcriptome study can reveal the functional genes involved in lignocellulose degradation and changes in gene expression patterns influenced by growth conditions.

### 4.3. Proteomics

Although a comprehensive analysis of genomic and metagenomic data can reveal the potential functional genes; this information often correlates poorly with protein expression or other strain phenotypes. For instance, the functional genes identified by genomic and transcriptomic data could not identify and correlate the concentration, function, and metabolic activity of expressed proteins (Xiao et al., 2017). The comprehensive metaproteomics approach provides a way to analyze functional proteins in microorganisms found in the natural environment and study complex metabolic pathways in greater functional depth (Bastida and Jehmlich, 2016).

Proteins expressed in biological systems can be measured using mass spectrometry (MS). Proteomics studies are used to evaluate changes in protein abundance, protein isoforms, post-translational modifications, enzyme activity, and their interactions in different environments. MS and MS/MS-based quantitative proteomic measurements can identify thousands of peptides or proteins present in the cellular environment and can be used to characterize these proteins. Metaproteomic analysis of *Streptomyces* sp. SSR-198 revealed that the proteins identified in its secretome consist of cellulases (30%), proteases (21%), other proteins (32%), and hypothetical proteins (17%) (Singh et al., 2015). In targeted proteomics analysis of the *Thermobifida fusca* secretome, the isobaric tagging approach for relative and absolute quantification (iTRAQ) was used along with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to evaluate the effects of cellulose and lignin substrates on changes in the abundance of proteins and its metabolic activities (Adav et al., 2010). Identifying and characterizing a protein involved in the de-polymerizing LCB, degradation of lignocellulose, and detoxifying metabolites will help design an efficient enzyme cocktail for the rapid fermentation of LCB at an industrial scale (D'haeseleer et al., 2013).

### 4.4. Metabolomics

Metabolic studies aim to identify and characterize low molecular weight substrates, intermediates, and end-products. In microbes, the metabolomics approach can be used to study intra- and extracellular metabolites produced during cellular processes (Roessner and Bowne, 2009; van der Werf et al., 2007). Metabolites generated during metabolic pathways can be detected by gas chromatography-mass spectrometry (GC-MS) and/or liquid chromatography-mass spectrometry (LC-MS) (van der Werf et al., 2007). A high-throughput nanostructure initiator mass spectrometry (NIMS) technique has been used in recent metabolomic studies on microbial communities (Cheng et al., 2013; Northen et al., 2007). In targeted metabolomics approaches, the structure of lignocellulosic substrates has been analyzed along with the products and intermediates formed during microbial hydrolysis of LCB (Klinke et al., 2004). Cellulose, hemicellulose, and lignin are hydrolyzed into an array of products, such as D-glucose residues, xylose, mannose, galactose, arabinose, levulinic acid, furfural, furfuryl alcohol, 2-furanmethanol acetate, HMF, and phenolic compounds, which can be identified using GC-MS (Zha et al., 2014). The targeted approach can be used to optimize pretreatment conditions, identify inhibitors and their characterization (Raj et al., 2007). The targeted metabolomics approach has been used in various detoxification studies to optimize the fermentation process and increase the product yield (Alriksson et al., 2011).

Recent advancements in metabolomics techniques have allowed identification and characterization of the intracellular and extracellular metabolites and aided the interpretation of strain metabolic pathways to understand their underlying mechanisms (Fig. 3c) (Calusinska et al., 2020). The cellular responses of *Clostridium acetobutylicum* during LCB hydrolysis help to understand the inhibitory effects of phenol, furaldehyde, and formic acid on carbohydrate metabolism and regulation modules functioning *in vivo* (Liu et al., 2019a). The systematic analysis of the effects of these inhibitors can aid the optimization of fermentation conditions and strain modifications.

## 5. LCB valorization *via* anaerobic bioprocesses

An innovative pretreatment method, along with efficient anaerobic bioprocesses, results in maximum digestibility and subsequent valorization of LCB, which helps to overcome the challenges encountered in efficient biofuel production (Nkemka et al., 2015). High-valued fermentative products of anaerobic bioprocesses, such as methane, hydrogen, ethanol, and biobutanol, can be obtained *via* photo and dark-fermentation, AD, ethanol fermentation, and ABE fermentation (Fig. 4).

### 5.1. Photo and dark fermentation

The sustainable production of bio-$H_2$ from LCB is preferred and intensively studied due to its higher energy yield (142 kJ/g), use of clean energy, more comprehensive industrial application, ease of control, easy conversion to electricity by a fuel cell, and operational safety (Basak et al., 2020a; Ndayisenga et al., 2021). Among the bio-$H_2$ production processes, photo-fermentation (light-dependent) or dark-fermentation (light-independent) offer a practical application for the conversion of various organic wastes and carbohydrate enriched wastewater into bio-$H_2$ under moderate operating conditions (Table 6) (Wang and Yin, 2017). In photo-fermentation, light and organic acids act as the energy source and electron donor, respectively, and can be utilized by purple non-sulfur (PNS) to generate bio-$H_2$ while catalyzing nitrogen fixation reactions using the nitrogenase (McKinlay, 2014). Microwave-assisted alkali pretreated rice straw (0.5% NaOH solution and 15 min microwave at 2.45 GHz and 1000 W to maintain the temperature at 140 °C) was enzymatically hydrolyzed to get 69.3% reducing sugars and subsequently used for bio-$H_2$ production of 155 mL/g total volatile solids (TVS) *via* dark fermentation. After fermentation, the residual nutrient medium, mainly containing acetate and butyrate, was further utilized by immobilized photo-fermentative synthetic bacteria to generate total bio-$H_2$ 463 mL/g TVS, which is 43.2% of the theoretical yield (Cheng et al., 2011). A few critical operating parameters, such as the pH, C/N ratio, and light intensity, critically affect the metabolic pathways of

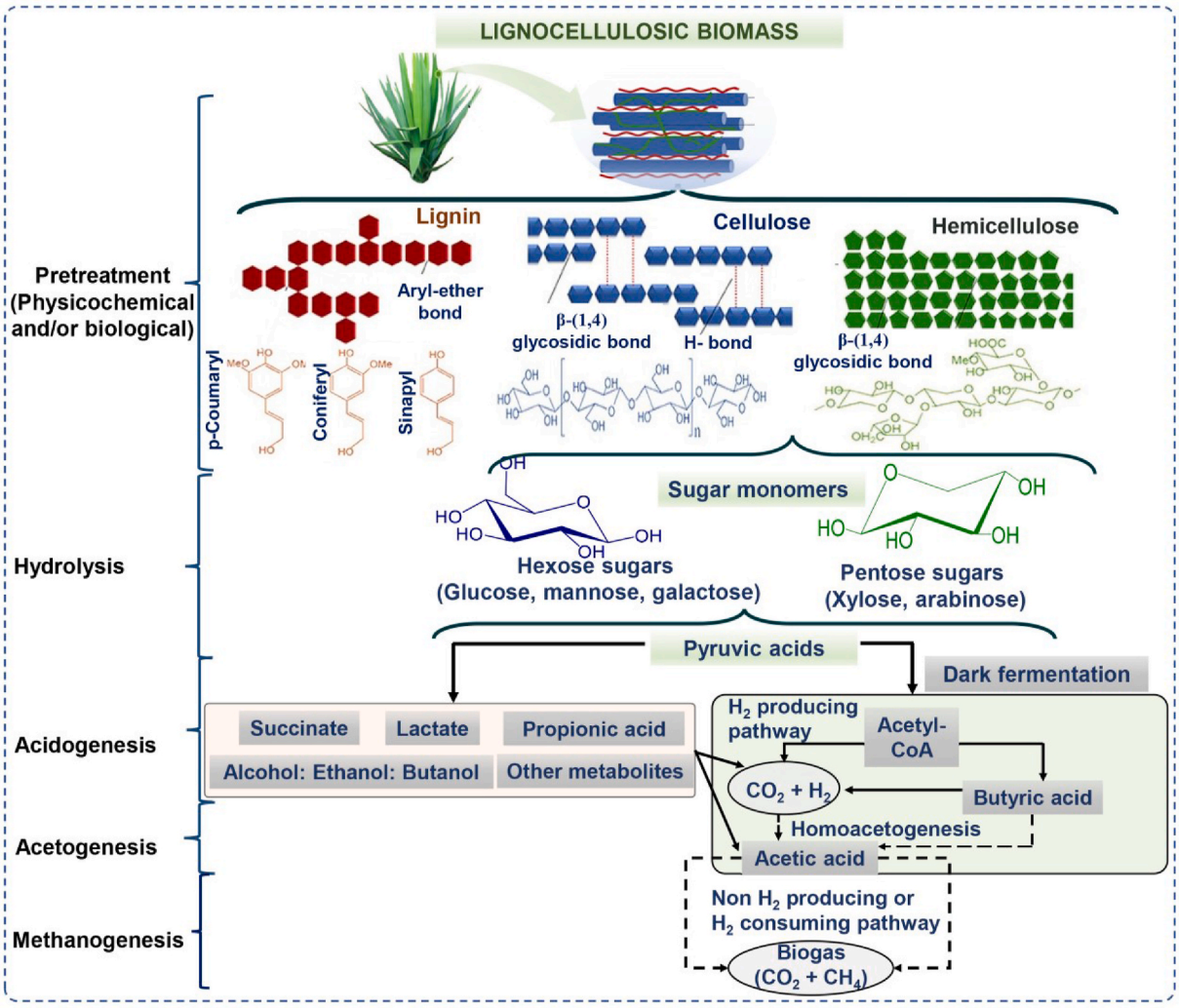


**Fig. 4.** Different anaerobic bioprocesses for the conversion of lignocellulosic substrates to biofuels [adapted from Monlau et al. (2013) with permission from Taylor & Francis, License No. 5181250532546; Baruah et al. (2018)].

photo-fermentative microorganisms. A low C/N ratio and high pH (>7.6) of the medium can affect microbial growth and damage the membrane of the microbial cells, resulting in microbial cluster formation. This leads to a decrease in the light conversion efficiency, which further decreases the yield and productivity of bio-$H_2$ (Keskin and Hallenbeck, 2012). Al-Mohammedawi et al. (2018) evaluated the optimum pH (7.4), C/N ratio (27.5), and light intensity (126 W/$m^2$) to obtain the maximum bio-$H_2$ productivity and potential of 41.7 and 960 mL/L, respectively, using *Rhodobacter sphaeroides* 158 DSM.

High bio-$H_2$ productivity using pure culture or synthetic co-culture or acclimatized microbiome can be achieved *via* the dark fermentation of reducing sugars (Chang et al., 2018). In addition, a diverse microbial consortium is required to produce bio-$H_2$ from lignocellulosic waste substrates, which makes the process economical as the system does not require sterilization (Basak et al., 2020a). However, competitors of bio-$H_2$ producers (such as lactate-producing bacteria) or bio-$H_2$ consumers (such as homoacetogens and hydrogenotrophic methanogens) may grow in the reactor, resulting in a drastic reduction of the overall bio-$H_2$ yield (Bundhoo and Mohee, 2016). Inoculum pretreatment by exposing mixed cultures to harsh environmental conditions (such as temperature and pH shocks) to select $H_2$-producing microbes, such as *Bacillus*, *Clostridium*, and *Thermoanaerobacterium*, which survives through endospore formation, can be a suitable strategy to sustain a high bio-$H_2$ yield (Galperin, 2013). For example, the acid pretreated inoculum was found to be most effective for mesophilic consortia (37 °C) with a relative abundance of *Clostridiaceae* to produce 0.8 mol $H_2$/mol of xylose. Similarly, alkaline soaked (24 h treatment at pH 10) thermophilic consortia (at 55 °C), with a relative abundance of *Clostridium* and *Thermoanaerobacterium*, may produce 1.2 mol $H_2$/mol xylose by repressing the growth of the *Lactobacillaceae* community (Dessì et al., 2018). However, bio-$H_2$ producing microbiota is highly susceptible to lignocellulosic inhibitors due to their low diversity, sensitive nature, short substrate retention duration, and a low ratio of inoculum to the substrate (Basak et al., 2020a). Thus, a suitable combination of pretreatment and inhibitor detoxification processes is necessary for the sustainable production of bio-$H_2$ using inexpensive lignocellulosic feedstocks *via* photo/dark-fermentation.

### 5.2. Anaerobic digestion

AD is the conversion of organic waste into valuable biogas *via* intimate syntropy among the cellulolytic/hemicellulolytic microbes, acidogenic or fermentative microbes, and methanogenic archaea bacteria which are responsible for hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The anaerobic co-digestion (AcoD), integrated or two-stage AD, and bioaugmentation have been identified as mainstream biological strategies as AD for biogas production from LCB (Tabatabaei et al., 2020b). The first step, hydrolysis, is the rate-determining step of

**Table 6**
Valorization of various pretreated lignocellulosic substrates by different anaerobic bioprocesses.

| Pretreatment methods and feedstocks | [a]Feedstocks composition before pretreatment | Operating conditions | Composition of biomass after pretreatment | Yield of TRS after saccharification | Yield and/or productivity of final products of anaerobic bioprocesses | Ref. |
|---|---|---|---|---|---|---|
| **Photo/dark-fermentation** | | | | | | |
| Combined ultrasound and dilute HCl acid, grass biomass | Soluble-COD, 272.8 mg/g-dry biomass; carbohydrates, 86 mg/g-dry biomass; CrI, 21 | HCl, 1% (w/w); ultrasound treatment at 260 W for 30 min and heated at 100 °C for 30 min | Enhanced soluble-COD, 541 mg/g-dry biomass; carbohydrate, 289.7 mg/g-dry biomass; crystallinity index, 40.8 | – | Bio-$H_2$ of 42.2 mL/g-dry grass by thermal treatment at 100 °C for 15 min in anaerobically digested sludge enriched with *Clostridium* sp. | Yang and Wang (2019) |
| Fungal, rice straw | C, 50.4%; H, 28.7%; L, 19.9% | Treated with edible fungus *Gymnopus contrarius* J2 for 15 d at room temperature | C, 38.8%; H, 16.2%; L, 11.6% | – | Maximum bio-$H_2$ produced 126.1 mL/g-substrate by *T. thermosaccharolyticum* DD32 | Sheng et al. (2018) |
| Ultrasound and acid, Cornstalk (CS) | – | Ultrasound for 1.5 h at 25 kHz in the presence of acid (2% $H_2SO_4$) | – | – | Enhancement of average $H_2$ production rate (17.03 mL/$g_{CS}$/h) and specific $H_2$ accumulation (142.59 mL/$g_{CS}$) of 6.1 and 9.3 times, respectively, in comparison to the group without treatment | Wang et al. (2012) |
| Ultrasound, palm oil mill effluent | – | Ultrasound at 20 kHz and 70% amplitude for 45 min | – | – | Increased bio-$H_2$ production from 467 mL to 872.4 mL | Budiman and Wu (2016) |
| **Anaerobic digestion** | | | | | | |
| Steam explosion, coffee husk | C, 29.17%; H, 28.96%; L, 17.67% | Steam under pressure at 120 °C for 60 min | Hydrolysate in the liquid stage contained 47.6% of C; 57.1% of H; 50.8% L in soluble form | – | Loading rate of F/M, 0.7 g of COD/g of $VS_{inoculum}$ to get the yield of 144.96 mL $CH_4$/g COD in a batch reactor at 35 °C for 25 d of fermentation | Baeta et al. (2017) |
| Hydrothermal, sugarcane bagasse | C, 44.2%; H, 23.6%; L, 25.8%; extractives, 2.2%; ash, 1.4% | Pretreatment at 178.6 °C for 43.6 min at 0.24 g/mL solid loading rate | Xylose, 18.4 g/L (80% of total sugars) from hemicellulose degraded hydrolysate | – | Maximum production of 270 mL $CH_4$/g COD in continuous up-flow anaerobic sludge blanket reactor at 20–30 °C for 168 d of fermentation | Ribeiro et al. (2017) |
| Hydrothermal, *Picea abies* (Norway spruce) | | Temperature of 140 °C for 300 min | Soluble COD, 12.6 g CODs/L; arabinose, 1.63 g/L; glucose, 1.55 g/L; xylose, 1.95 g/L; mannose, 5.11 g/L | – | Maximum production of 266 mL $CH_4$/g COD in 103 d in batch 100 mL syringes at 55 °C | Ghimire et al. (2021) |
| Hydrothermal, Napier grass (*Pennisetum purpureum*) | C, 40.1%; H, 32.3%; L, 10.2% | Temperature of 175 °C and 113 psi for 15 min | C, 48.5%; H, 11.3%; L, 14.7% | – | Methane yield of 238.2 mL $CH_4$/g VS in 42 d of batch study | Phuttaro et al. (2019) |
| Ultrasound and alkali (NaOH), corn stalk | C, 36.4%, H, 30.3%; L, 6.9%; TS, 90.3%; VS, 87.7% | Dual-frequency sonication, i.e., 57 kHz and 20 kHz at 50 W and 2% NaOH for 36 h of treatment time using 52 g of feedstock | – | – | Combined pretreatment enhanced 56.5% biogas yield, 71.4% TS removal, 77.1% VS reduction, and 19.7% net energy compared with the untreated biomass | Dong et al. (2018) |
| **Ethanol fermentation** | | | | | | |
| Alkaline hydrothermal, *Brachiaria mutica* (Para grass) | C, 42%; H, 20%; L, 19% | NaOH, 0.5% (w/v); $H_2SO_4$, 2% (w/v); autoclaved at 121 °C for 1 h | C, 58%; H, 22%; L, 10% | Biomass recovery, 20%; 696 mg/g TRS in 48 h (cellulase saccharification) | Ethanol yield of 71.8% in 72 h | Sahoo et al. (2017) |
| Ultrasound and dilute acid, *Typha angustifolia* | Solid, 96.67%; C, 43.96%; L, 19.27%; ash, 3.39%; extractive (aqueous), 30.57% | Biomass loading, 10% (w/v); sulphuric acid, 1% (w/w); ultrasound, power, 70% and 24 kHz at 60 °C for 60 min | Solid 93.47%, C, 68.73%; L, 17.53%; ash, 3.18%; extractive (aqueous), 25.61% | Maximum TRS 299.74 mg/g by enzymatic saccharification | Ethanol yield of 81.9% | Paramasivan et al. (2021) |
| Alkaline hot-water treatment, *Pennisetum purpureum* (Elephant grass) | C, 22.6%; H, 20.9%; L, 19.4% | NaOH 2%, 120 °C for 1 h | C, 22.6%; H, 21.9%; L, 6.8% | Biomass recovery, 30%; TRS, 146.9 mg/g in 45 h (cellulase saccharification) | Ethanol yield of 95% | Eliana et al. (2014) |
| Plasma-assisted ozonolysis, wheat straw | L, 19.2%; glucan, 41.6%; xylan, 23%; arabinan, 3.5%; ash, 5.5% | A dielectric barrier discharge was used to convert air to ozone at atmospheric pressure, | L, 9.5%; glucan, 47; xylan, 18; arabinan, 2; ash, 6 | Material loss of 1.6%, enzymatic saccharification yielded 78% sugars after 48 h | Ethanol yield of 53% | Schultz-Jensen et al. (2011) |

(*continued on next page*)

**Table 6** (*continued*)

| Pretreatment methods and feedstocks | [a]Feedstocks composition before pretreatment | Operating conditions | Composition of biomass after pretreatment | Yield of TRS after saccharification | Yield and/or productivity of final products of anaerobic bioprocesses | Ref. |
|---|---|---|---|---|---|---|
| | | 50% dry biomass slurry was treated by ozonolysis for 2 h | | | | |
| Microwave and hydrothermal in dilute acid solution, rye stillages | C, 16%; H, 29.6%; L, 15.57% | Microwave at 300 W for 15 min under 0.37 MPa hydrothermal pressure in 0.2 M $H_2SO_4$ solution | C, 89.2% | Enzymatic saccharification in 24 h yielded TRS of 156 mg/g | Ethanol concentration of 20 g/L in 48 h after detoxification of toxic stress (HMF) with activated carbon | Mikulski and Kłosowski (2020) |
| **ABE fermentation** | | | | | | |
| Autohydrolysis, sweet sorghum bagasse | C, 34.85%; H, 37.32%; L, 27.36%; ash, 1% | Biomass to liquid ration, 8% (w/v), 210 °C for 60 min; pH, 3.4; activated charcoal, 2.4% (w/v) added to hydrolysate | Xylose, 18.12 g/L; glucose, 4.3 g/L; xylo-oligomers, 11.93 g/L; sweet sorghum grain, 20 g/L | – | Co-digestion of sorghum bagasse with sorghum grain powder by *C. acetobutylicum* NRRL B-591 produced butanol, 5 g/L; acetone, 3 g/L; ethanol, 0.3 g/L | Mirfakhar et al. (2020) |
| DESs, corn stover | C, 31%; H, 20.1%; L, 25.4%; ash, 8.6% | ChCl: formic acid ratio 1:2 in acidic solution | C, 47.9%; H, 9.8%; L, 27.9; ash, 7.1 | Enzyme dosage 25 FPU/g at 130 °C for 3 h yielded 17 g/L of glucose | Butanol, 5.63 g/L; yield, 0.17 g/g; productivity, 0.12 g/L/h by *C. saccharobutylicum* DSM 13864 | |
| Aqueous ammonia, dilute acid and oxidative ammonolysis, sugarcane bagasse | Glucan 41.7%; acid insoluble L, 22.53% | Combined 5% $NH_3H_2O$ at 140 °C for 1 h and biomass loading, 1:10 (w/v) in a 0.5 L high pressure reactor | Glucan, 61.4%; acid insoluble L, 33.7% | Enzymatic saccharification by cellulase and xylanases at 50 °C with substrate loading (2%, w/v) yielded glucose (29.6 g/L) and xylose (1.16 g/L) | ABE fermentation yielded acetone (3.84 g/L), butanol (7.68 g/L), and ethanol (0.6 g/L) in 120 h by *C. acetobutylicum* | Li et al. (2017) |
| Steam explosion, rice straw | C, 32–47%; H, 19–27%; L, 5–24%; ash, 18.8% | Steam under the pressure of 0.103 MPa at 121 °C for 30 min | – | Enzymatic hydrolysis of 1% acid hydrolyzed pretreated biomass at 60 °C for 24 h yielded TRS 380 mg/g | Highest ABE production, 2.07 g/L; productivity, 0.017 g/L/h; yield, 41.4 g/kg raw rice straw by *C. acetobutylicum* MTCC 481 by anaerobic fermentation for 120 h | Ranjan and Moholkar (2013) |
| Microwave and hydrothermal, Brewer's spent grain (BSG) biomass | C, 17.9%; H, 28.7% (xylan, 20.7% and arabinose, 8%); L, 25.8% | Solid loading, 10% (w/v); microwave-hydrothermal at 192.7 °C for 5.4 min | C, 26%; H, 77%; L, 43.3% in solid fraction; total sugars, 26 g/L; inhibitors, 2.2 g/L are present in the liquid fraction | 70% glucose recovery after enzymatic hydrolysis | Butanol, 8.3 g/L; yield; 46 kg/t BSG using *C. beijerinckii* | López-Linares et al. (2019) |

[a] C, cellulose; H, hemicellulose; L, lignin.

the LCB-based AD process and remains a bottleneck in the effective utilization and commercial application of LCB in large-scale biogas projects (Saha et al., 2019b; Shrestha et al., 2017). Biological pretreatment using hydrolytic microorganisms or consortia with strong lignin degradation abilities can shorten the anaerobic fermentation time, enhance the bio-digestibility, and increase the bio-methanation rates (Tabatabaei et al., 2020a). In addition, an imbalance in the C/N ratio in the feedstock is another challenge for the anaerobic conversion of LCB to biomethane (Saha et al., 2018).

To overcome the substrate-inherent limitations, substrate-specific pretreatment and/or substrate-focused AD optimization methods by adding complementary nutrients can prepare a more adaptable AD-microbiome for maximum AD performance (Kurade et al., 2019; Saha et al., 2019a). Blending of different organic matter, such as animal manure, pig urine, dairy manure, lipidic, municipal, and kitchen wastes with LCB during AcoD improved the methane yield by 4-fold as compared to mono-digestion, mainly due to process stabilization as well as element and nutrient balance (Hagos et al., 2017; Salama et al., 2019). Firmicutes, especially, *Bacteroidetes*, *Actinobacteria*, and *Clostridiales* are observed as dominant phyla during AcoD (Mata-Alvarez et al., 2014). It offers several advantages, such as high pH stability, diluting inhibitory compounds, and providing an effective starter inoculum for the new AD process. However, the operational difficulties in conventional single-stage co-digestion restrict the maximum conversion of organic waste biomass into methane due to acidification of the reactor by rapid formation of short and/or medium-chain fatty acids, resulting in a decrease of pH beyond the critical level for methanogenic activity (Kurade et al., 2020). Currently, only 10% of large-scale commercial plants in Europe are AcoD-based with the prime substrate as animal manure and agro-industrial along with municipal solid wastes used as co-substrate (Mata-Alvarez et al., 2014).

The application of acclimatized AD microbiota before adding substrates in anaerobic two-stage co-digestion is beneficial to counteract the system failures and instability, reduce the impact of inhibitory compounds, achieve better substrate digestion, and higher gas productivity (Kang et al., 2021). Two-stage AD system consists of the acidogenic fermenter and methanogenic digester to provide optimal process conditions, balance acidity and hydrogen level pressure for the smooth growth of respective microbiota, and enhance energy recovery as biohythane ($H_2 + CH_4$) from the organic feedstocks (Castellano-Hinojosa et al., 2018). The thermophilic microorganisms have shown high activity in low pH and thermophilic conditions to produce volatile fatty acids, which inhibits the methanogens in the acid reactor. The next stage, i.e., methanogenic digester, provides mesophilic conditions and neutral pH to bring efficacy and species diversity in methanogenic microorganisms. Basak et al. (2020b) reported 47% (214.1 mL/g $VS_{added}$) higher energy recovery as biohythane in two-stage AD using fungal (*Aspergillus fumigatus*) pretreated polysaccharide waste biomass.

Bioaugmentation with engineered lignocellulolytic microbiomes, i.e., the addition of microbial consortium with potent lignocellulolytic activities during AD, is a promising strategy to increase the digestion of LCB for a higher yield of biomethane during the start-up period at a commercial scale (Tabatabaei et al., 2020b). Such a strategy helps to restore the reactor performance, enhancing the conversion rate of lipidic and LCB waste and reducing the lag phase during the start-up period of the new system (Tale et al., 2015). The bioaugmented AD system has

shown microbial communities with a large CAZyme secreting population in Bacteroidetes and Firmicutes compared to the indigenous microbiome in conventional AD (Basak et al., 2022). Enhanced sorghum biomass degradation (27%) and increased biomethane yield (20%) along with a stable AD-microbiome could be achieved using cellulolytic and methanogenic consortia from natural sources, such as cow and goat rumen content (Ozbayram et al., 2018). Applying natural microbial consortia in a newly designed bionic reactor based on the ruminant stomach produces 21.6 mL/g VS/day and 256.5 mL/g VS from corn stover, as productivity and cumulative production of biogas, respectively (Table 6) (Zhang et al., 2014).

### 5.3. Ethanol fermentation

Bioethanol obtained from renewable biomass, especially LCB, is considered a promising alternative to fossil fuels to achieve sustainable development goals by reducing the emissions of CO, hydrocarbons, and GHGs (Kumar et al., 2019). Therefore, bioethanol production has attracted global attention, such as the European Union (EU) has set a target of 3.5% LCB-based bioethanol blended transport fuel by 2021 (Padella et al., 2019). However, compared to the sugar- or starch-based bioethanol production processes, a more complicated and expensive process consisting of four different steps: pretreatment, saccharification, fermentation, and purification, is required to produce lignocellulosic bioethanol (Haghighi Mood et al., 2013; Morales et al., 2021). Moreover, severe pretreatment conditions, including high temperature and pressure, and the presence of concentrated acids or bases, lead to the production of toxic by-products that affect the microbial fermentation process, resulting in a low yield of bioethanol (Yuan et al., 2021). Ultrasound-assisted pretreatment under mildly acidic conditions can delignify LCB (*Saccharum arundinaceum*) and expose the cellulose for subsequent saccharification and fermentation, yielding up to 309 mg/g-TRSs and 78% ethanol, respectively (Table 6) (Paramasivan et al., 2021).

Consolidated bioprocessing (CBP) has been suggested as a fermentation strategy for obtaining biofuels from pretreated LCB, where a combination of two or more microorganisms performs saccharification and fermentation in a bioreactor (Mohapatra et al., 2020). In contrast, other approaches, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF), need multi-steps for the cellulose production, hydrolysis, and fermentation for ethanol production (Agbor et al., 2014). Based on the limitation of two-step processes in SHF, integrated approaches, such as SSF and SSCF, saccharification and fermentation are conducted in a single reactor, thus allowing low operation cost and less inhibitor generation. But different process conditions for an enzyme (for hydrolysis) and microorganism (for fermentation) and faster conversion of hexoses than pentoses are critical issues related to SSF and SSCF processes (Vohra et al., 2014). The CBP approach is lucrative for the bioprocessing of cellulosic biomass using the ingeniously designed microbial consortia, which has desirable aspects related to higher stability, better operating functions, and enhanced productivity than using a single organism (Xia et al., 2016a). In addition, synergistic labor division is possible among ingenious designated microbial consortia, either in cooperation or competitive mode, to perform complex tasks, resulting in economic bioethanol production at approximately 41% lower cost than conventional methods (Liu et al., 2019b). A moderate temperature (50–70 °C) for the saccharification of pretreated LCB is required during CBP to achieve the efficient performance of cellulolytic enzymes. Therefore, high-temperature resistant anaerobic thermophiles prefer microorganisms over conventionally used yeast cells for CBP for biofuel production. Furthermore, anaerobic thermophiles, such as *Clostridium* and *Caldicellulosiruptor*, can efficiently utilize monosaccharides (C6 and C5) and are mainly resistant to inhibitors during fermentation (Singh et al., 2017b).

CBP represents an economical and ideal approach for second-generation biofuel production by utilizing a broad spectrum of lignocellulosic substrates without the addition of costly exogenous enzymes (Lugani et al., 2020). Developing pilot-scale and industrial-scale processes using CBP in single vessel and temperature are much simpler than SHF and SSF due to differences in the operating temperatures during saccharification and fermentation steps (Agbor et al., 2014). However, low ethanol tolerance ($\leq$2%, v/v) of thermophilic microbes shows a significant obstacle in the industrial-scale application (Rastogi and Shrivastava, 2017). Thus, a suitable CBP-enabling microorganism is a major quest that limits the process technology design for large-scale production of low-cost fuels compared to sequential step processes (SHF and SSF). The synthesis of robust engineered microorganisms using synthetic biology, cell-surface engineering, and metabolic engineering seems to be promising approaches to improve ethanol production through CBP (Kazemi Shariat Panahi et al., 2021; Lugani et al., 2020). Yanase et al. (2010) genetically engineered a thermotolerant yeast strain *Kluyveromyces marxianus* by co-displaying endoglucanase (from *Trichoderma reesei*) and $\beta$-glucosidase (from *Aspergillus aculeatus*) on the cell surface, which grew well at a temperature of 48 °C and yielded 0.47 g/g of carbohydrate. Such development of the recombinant yeast strain shows a significant advancement towards the realization of CBP.

### 5.4. ABE fermentation

The transformation of LCB through developing novel biorefinery designs and technologies for alternative biofuel and chemical production leads to chemical process sustainability, which has gained worldwide attention. Butanol is one of the established alternative biofuels, which can be produced *via* ABE fermentation technology (Meramo-Hurtado et al., 2021). ABE fermentation is a strictly anaerobic process completed in two phases: acidogenic and solventogenic phases. During the acidogenic phase, butyric and acetic acids, and gases ($CO_2$ and $H_2$) are formed, accompanied by a drop in the pH of the medium. In the solventogenic phase, the acids are re-assimilated to form ABE solvents along with pH restoration (Capilla et al., 2021). Widely known ABE bacteria from the class *Clostridia* can consume various carbon sources (such as sugars, whey, starch, cellulose, and hemicellulose) to produce second-generation biofuels (Zetty-Arenas et al., 2019). However, the conversion of LCB to fermentable monomeric sugars *via* various physicochemical and biological methods is the rate-limiting step in obtaining inexpensive and sustainable feedstocks for ABE fermentation. Autohydrolysis by water is an economical and eco-friendly pretreatment process to release the reducing sugars of hemicellulose from LCB, which can be an ideal feedstock for *Clostridia* for butanol fermentation without generating inhibitors. Use of DESs (choline chloride: formic acid), an effective and biocompatible pretreatment method, was proposed for the pretreatment of corn stover biomass and released 17.0 g/L (99% yield) glucose *via* enzymatic saccharification and subsequently produced 5.63 g/L butanol with a yield of 0.17 g/g and productivity of 0.12 g/L/h using a novel strain *C. saccharobutylicum* DSM 13864 (Table 6) (Xu et al., 2016). *C. acetobutylicum* cannot ferment hemicellulose-derived sugars (mainly xylose) as the sole carbon source due to its carbon catabolite repression mechanism (Ren et al., 2010). To completely utilize LCB in ABE fermentation, different approaches, such as the co-fermentation with starch and the application of genetically modified microorganisms (Ren et al., 2010), have been adopted to enhance butanol production and butanol tolerance simultaneously.

## 6. Sustainability aspects of valorization of LCB into biofuels

The sustainability aspects of different methods used for the valorization of LCB can be judged by techno-economic evaluation, life-cycle assessment (LCA), environmental impact assessment, and thermodynamic-based (emergy/energy/exergy) approaches (Soltanian et al., 2020). The energy analysis provides information about energy

efficiency considering only energy quantity. In contrast, exergy includes both, quality and quantity of energy flow in a system following the first and second law of thermodynamics (Aghbashlo et al., 2019). The exergy analysis measures in terms of exergy destruction linked to economic loss and resources degradation of the system under consideration, thus making a good correlation with GHGs emission (Soltanian et al., 2019). LCA could measure and quantify the environmental impacts of the final product in the energy system, but difficult to evaluate environmental consequences at the component level or intermediate products. These limitations can be fixed mainly by integrating LCA and economical with exergy-based approaches, such as exergoenvironmental and exergoeconomic analysis tools, which provide vital information about hot spots of thermodynamic inadequacy, techno-economic feasibility, and impacts on the environment during each flow and an intermediate component of lignocellulosic biofuels production system (Aghbashlo et al., 2021; Fallahi et al., 2021).

The environmental impact during biofuels production depends upon the types of feedstock and process conformation. For example, methanol production using corn as a feedstock has shown a negative emission of 0.99 kg $CO_2$-eq/kg $CH_3OH$ in comparison to potential global warming value of 2.9 kg $CO_2$-eq/kg $CH_3OH$ for coal-based production (Kajaste et al., 2018). O'Connor et al. (2020) demonstrated surplus energy of 73–79% generated along with net reduction of $CO_2$ ranging in-between 2059 to 173,237 kg $CO_2$-eq/yr through small-scale AD plants using livestock manure and municipal organic waste from Irish dairy farms with herd sizes greater than 100 cows. The utilization of biomass-derived fuels results in zero net $CO_2$ emissions due to the biogenic nature of $CO_2$ emitted during their generation and consumption (Mandley et al., 2020). However, various factors, such as the composition of the feedstock, pretreatment method enzymatic saccharification or microbial hydrolysis, fermentation/AD process, and downstream recovery, have significantly affected economic feasibility, envirosafety, and sustainability during lignocellulosic biofuels production (Zabed et al., 2019).

The pretreatment process has been identified as a critical step in impacting sustainable, economic, and environmentally benign biofuel production due to its chemical-dependent and energy-intensive nature (da Silva et al., 2016). SEP was assessed as most exergy-efficient, low environment burden, and high index of sustainability approach for lignocellulosic ethanol production from sugarcane bagasse using SSF process, followed by organosolv and dilute acid methods (Ojeda et al., 2011). Hoang et al. (2021) evaluated microwave-based heating as faster and energy-efficient method that could reduce 10-fold in pretreatment time while consuming 22.5-fold less energy than conventional heating. The energy consumption was reduced from 560 kJ to 192 kJ by switching the oil-bath technique to microwave-assisted pretreatment in IL-based (1-ethyl-3-methylimidazolium acetate) wood delignification (Wang et al., 2013). The microbial pretreatment method was investigated as the highest exergy efficient process (90.9%) during oil-palm processing due to relatively less energy and chemicals consumption than SEP and organosolv processes (Ofori-Boateng and Lee, 2013). The biochemical composition, especially the lignin content of LCB, has also played a significant role in the exergy efficiency of pretreatment processes (Ortiz and de Oliveira Jr, 2014). Further, downstream saccharification and fermentation pretreated sugarcane bagasse using SSF configuration outperformed the SHF approach in exergy efficiency during bioethanol production (Ortiz and de Oliveira Jr, 2016). Overall, future studies need to investigate on exergoeconomic and exergoenvironmental aspects of various unexplored pretreatment methods to lower the LCB pretreatment cost while reducing the exergy destruction cost of saccharification and/or fermentation subsystem (Soltanian et al., 2020).

## 7. Conclusions and future perspectives

Bioenergy production from LCB *via* anaerobic bioprocesses is vital for the sustainable development of society. However, a critical bottleneck in LCB bioprocessing is its pretreatment to make its recalcitrant structure readily biodegradable. Several studies have evaluated various biological and non-biological strategies for the effective pretreatment of LCB; however, the conventional processes still pose several limitations. Therefore, apart from the fine-tuning of existing technologies, there is a need to develop novel pretreatment methods to improve the sustainability and economic viability of biofuel production. Based on our extensive literature review, several critical aspects are summarized below that may be considered to overcome the current challenges for the effective valorization of LCB.

i. In-depth understanding of various intrinsic properties of LCB, including its dielectric properties, moisture content, presence of hydroxyl groups, crystalline/amorphous structures, and other features, such as the characteristics of lignin and its interactions with cellulose and hemicellulose, is necessary to ensure the selection of the appropriate pretreatment method.
ii. To overcome the challenges of low yield of fermentable carbohydrates, high energy consumption, discharge of polluting wastewater, and release of inhibitory compounds, it is crucial to develop more advanced pretreatment methods. Few emerging physicochemical pretreatment methods with mild/no use of chemicals, including microwave, ultrasound, DESs, irradiation, ozonolysis, SC-$CO_2$, and SEP show great potential for the efficient valorization of LCB. However, these pretreatment methods have specific pros and cons related to eco-friendliness and cost-effectivity for the complete delignification or effective biomass utilization.
iii. Microbial consortia used for anaerobic bioprocesses are vulnerable to lignocellulosic inhibitors. Therefore, it is necessary to detoxify these toxic compounds using cost-effective, technologically sound, and highly efficient methods. Adsorption-based detoxification using porous, activated carbon may be one such effective method to ensure the sustainable valorization of LCB *via* microbial fermentation.
iv. Biological pretreatment of LCB using cellulolytic bacteria, including Bacteroidetes, Firmicutes (mainly *Clostridia*), and fungi (especially white-rot fungi), exhibit great potential for hydrolyzing cellulose and hemicellulose; however, their involvement in lignin biodegradation remains unclear. The lignocellulolytic microbial communities showing excellent capacities for LCB biodegradation *via* their lignocellulolytic enzymatic machinery can be further assessed using the combination of omics approaches to develop effective biological-based strategies to enhance the hydrolytic efficiency. Furthermore, bio-mimicking the natural ecosystem, co-digestion, and natural microbial consortia are potential alternatives for enhanced LCB biodegradation.
v. The efficacy of various anaerobic bioprocesses is debilitated due to the low bioavailability of LCB for bioenergy conversion. Therefore, a comprehensive understanding of the recalcitrant nature of LCBs and their efficient pretreatment methods is the key to improving the overall productivity, yield, and economics of bioenergy production through various anaerobic bioprocesses.

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## Declaration of competing interest

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

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