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Recent advances on lignocellulosic bioresources and their valorization in biofuels production: Challenges and viability assessment

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ABSTRACT

Lignocellulosic biomass (LCB) waste materials are abundant in nature, and because of their high cellulose content, they rank among the most widely accessible and preferred feedstocks for the development of cost-effective biorefineries. The main obstacle to the long-term viability of this waste valorization at the pilot size, however, is the complexity of the structural composition of these wastes and the lack of a suitable bioprocess for their economical and efficient biotransformation. The current review investigates the potential for economically viable and environmentally friendly biotransformation of LCB wastes into cellulolytic enzymes and biofuels generation technologies. The review focuses on the efficient synthesis of enzymes and energy from LCB wastes through biotransformation. Based on the update progress, the information of the complexity constraint that currently exists in the LCB structure and the successful limitation surmounted have also been evaluated. To improve the overall bioprocess on a sustainable scale, other possible sustainable recommendations have also been proposed. Such LCB waste valorizations can contribute to the circular economy for sustainable future applications.

1. Introduction

The continual use and short lifespan of fossil fuels, which are the primary cause of energy crises, has resulted in a significant growth in environmental damage. Other significant global contributing variables include the rapid population

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growth and strong economic expansion. On a global scale, energy consumption has climbed by 418 EJ in 2019 and is predicted to increase by a further 516 EJ by the year 2040, which is equivalent to a 23% increase (Wang et al., 2021). Additionally, it has been reported that 33 Gt of CO_2 emissions occurred in 2019. According to the International Energy Agency (IEA), the carbon footprint will remain at 33 Gt in 2040, and in order to reach the goal of net zero CO_2 emissions in 2050, it is essential to produce and develop energy resources (Monir et al., 2021). Finding sustainable and affordable energy sources is one of the primary research areas being pursued worldwide to address these problems. One of the most promising and effective options in this scenario is the production of biofuels from LCB wastes. The most sustainable solution to address issues related to the global energy crises and climate changes is to use LCB as a viable feedstock for the development of biofuels (Seo et al., 2022). The use of these LCB residues also has the potential to benefit rural economies and the environment by preventing the direct dumping and burning of these wastes in open fields, which is the main source of environmental degradation. Though the topic is challenging and constantly looking for robust substitutes due to high production costs and a lack of acceptable alternative bioprocesses (Monir et al., 2020).

Additionally, the utilization of these waste residues includes the efficient production of cellulose-digesting enzymes, which are essential for the conversion of cellulosic biomass into biofuels and are currently one of the most researched topics (Bilal and Iqbal, 2020; Bukhari et al., 2019; Sampath et al., 2020). The main LCB cell wall constituents, cellulose, hemicellulose, and lignin, are the most prospective worldwide bioeconomy contributors. Since more than 200 sustainable biochemical and bioproducts have been made from LCB waste, the key area of concern is the development of low cost manufacturing techniques for these products (Ding et al., 2019; Kuila and Sharma, 2017; Nwamba et al., 2021). Among these bioproducts, the fermentation pathway of biofuel production *via* LCB digestion plays a crucial role in the sustainability of society due to its renewable, economical, pollution-free, and biological characteristics. Herein, microorganisms play a significant role in the development of fermentative biofuels and cellulose digesting enzymes (Adegboye et al., 2021).

Cellulases are system enzymes that work together to produce the necessary hydrolysis of cellulosic biomass. For the successful digestion of cellulosic biomass, synergistic action of all cellulolytic enzyme subcomponents is essential. Because cellulases have been developed for purposes other than cellulose hydrolysis on industrial platforms, demand for this enzyme is constantly on the rise, driving up production costs (Ferreira et al., 2021; Rodrigues and Odaneth, 2021). The need for cellulase to develop biomass-based biofuels is expected to increase steadily over time, replacing 30% of fossil fuels with sustainable fuels by the year 2025 for sustainable applications. Based on a similar pattern, the global growth promotion initiatives backed by a number of nations are predicted to help the biofuels industry reach over USD 950 million by 2024. Furthermore, it has been observed that enzyme applications have already dominated the commercial market as of 2021 and are still expanding to produce 2G biofuels and cost-effective enzymes using LCB waste (Deng et al., 2019; Ejaz et al., 2021; Tiwari et al., 2018).

As a result, the main goal of this review is to investigate the valorizations of LCB waste into the production of cellulolytic enzymes and biofuels, which are the two most significant and rapidly expanding industrial uses of these wastes. The study discusses cellulase enzymes, their LCB-based manufacturing and bioconversion efficiency, as well as their use in the generation of biofuels. The valorizations of LCB for cellulase and biofuels have been discussed, highlighted, and exposed in detail. Additionally, the current constraints and future directions have been discussed in relation to the production of biofuels, low cost industrial enzymes, and sustainable environmental implications.

2. Lignocellulosic biomass: structural overview

Lignocellulosic biomass is categorized as highly cellulose-populated biomass that also contains hemicellulose and lignin, two essential parts. The percentage of cellulose in total biomass is typically between 40 and 60 percent, with the remaining percentages being hemicellulose (20–40 percent), lignin polymer (10–25 percent), a little amount of pectin, and minerals (Tayyab et al., 2017). The individual unit in the cellulosic polymer structure is bounded by beta-glucosidase bonding, whereas in the linear structure, equal structures of hydroxyl groups presented on both sides supported the crystal structure of cellulose in parallel alignment and maintained it at the nanoscale, which is known as microfibrils (Kamm et al., 2017). These fibrils provide strength to the cellulosic structure by forming hydrogen bonds between the hydroxyl groups. Furthermore, the amorphous structure of cellulose refers to the less compact structure of cellulose, which is 3–30 times easier to degrade. Further, like cellulose, the other companion polymer called hemicellulose is made up of the monomeric sugars glucose, pentose, xylose, arabinose, and mannose, and due to its irregular structure, only an amorphous functional region is found in hemicellulose (Mitani, 2018; Paz-Cedeno et al., 2022; Yue et al., 2018) [Fig. 1].

Further, the number of units is also less than cellulose in hemicellulose's structure and accounts for 150–200 smaller units than cellulose; thus, hydrolysis of hemicellulose into monomeric sugars is easier than hydrolysis of cellulose (Rezania et al., 2020). Furthermore, the hydrophobicity of LCB is maintained by lignin, an aromatic polymer composed of three major phenolic subunits: p-hydroxyphenyl, guaiacyl, and syringyl. The structures of cellulose and hemicellulose are bonded with an ester bond, which forms a rigid structure and protects the polymer from external attack such as microbial, enzymatic, and chemical hydrolysis (Sperandio and Ferreira Filho, 2019).

Out of the 181.5 billion tons of LCB that are produced each year, 8.2 billion tons are readily available and used for roughly 42% and 43% of the total LCB from grass land and forest, respectively (Song et al., 2021). Among various and hugely available LCB, corn is the highest producing grain globally, and its annual global production is estimated at 1 billion

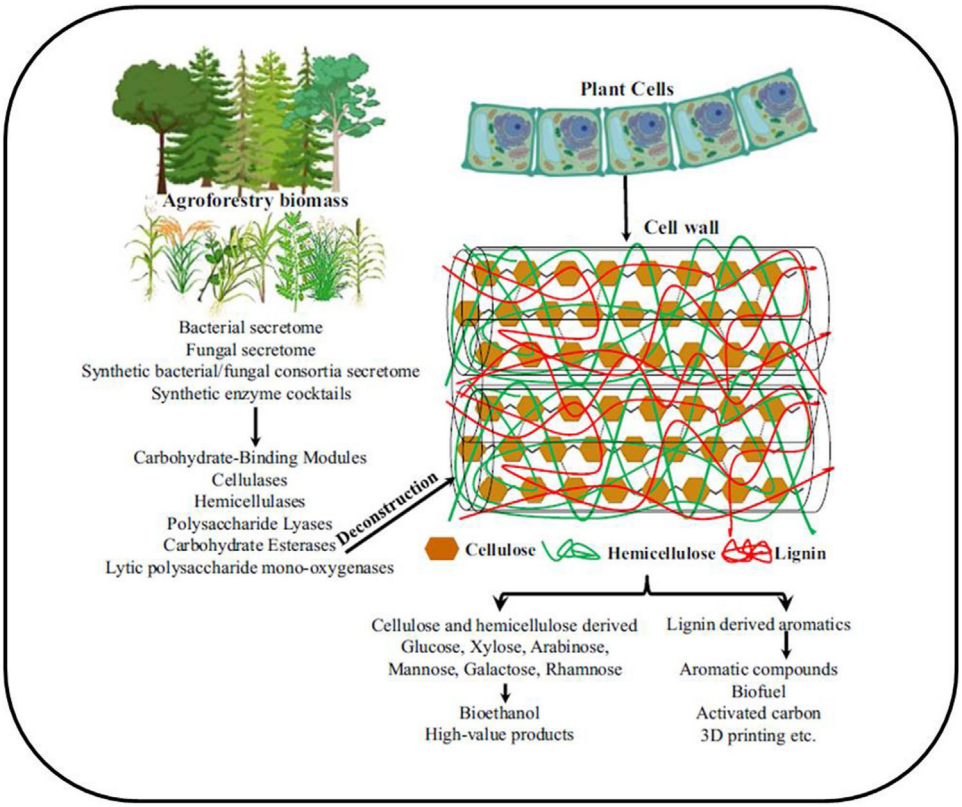

Fig. 1. An overview of biomass composition and degradation. *Source:* Adapted from Sethupathy et al. (2021) Open access CCBY 4.0.

tons, while production of wheat is around 529 million tons annually, followed by rice, which is around 731 million tons produced annually and is the major crop of Asia (Erenstein et al., 2022; Rodionova et al., 2022). Apart from these, other LCB are also generated in huge quantities; for example, the annual global production of sugarcane bagasse is 540 million tons, while the processing of palm oil produces, annually, 75 million tons of waste in the form of empty fruit bunches, mesocarp fiber, and palm kernel shell. Additionally, woody LCB is one of the key sources for biorefining applications due to advantages like low ash content, higher lignin content, season independence, and higher bulk density (Rodionova et al., 2022; Yan et al., 2017). Due to these efficient properties, LCBs are the ideal representative of biorefining applications and the valorization of these kinds of waste into developing value added products for environmental sustainability. However, substrate type, pretreatment, and identification, as well as the reduction of inhibitors, are key points that need to be addressed thoroughly to maximize the biotransformation of LCB waste into allied biorefining products such as enzymes, sugars, and biofuels (Deng et al., 2019; Sulzenbacher et al., 2023; Zhuo et al., 2018). Several pretreatment methods have been developed and established for the delignification of LCB waste, and to date the alkaline pretreatment method has been documented as the most promising and effective approach. In the study of Valles et al. alkaline pretreatment using NaOH has been regarded as the most effective pretreatment method for rice straw, and under the influence of optimum conditions, at 5% w/v of solid loading along with 0.75% w/v NaOH at 134 °C for 20 min, enhanced biomass production was 77.6 g kg RS^{-1} (Valles et al., 2021). Moreover, a maximum butanol titer of 10.1 g L^{-1} could be produced in the fermentation of a 72-h reaction. Similarly, in the research investigation of Tsegaye et al. 71.29% of lignin removal was recorded using 7.0% of alkali pretreatment of rice straw with NaOH (Tsegaye et al., 2019a). Rizal et al. confirmed the benefit of the alkaline pretreatment method for SCB pretreatment, which was best achieved using NaOH in 1.5 h and 77.26% more delignification was obtained than a control in a reactor volume of 120 L (Rizal et al., 2020). In the investigation of Jin et al. sequential pretreatment methods by applying NaOH as well as hydroxymethylation pretreatment (AHP) have been used in SCB biomass for improving saccharification (Jin et al., 2020). Under optimum conditions, glucose and xylose increased from 53.3% to 68.88% and 67.8% to 74.7%, respectively, resulting in 13% more ethanol concentration than the control after 24 h of fermentation. Moreover, alkaline pretreatment with NaOH is also regarded as a prominent pretreatment method by Tsegaye et al. (2019b). In the key results, 69.5% delignification along with the release of 72.67% of cellulose was noticed with 10% NaOH pretreatment at 80 °C up to 4 h before enzymatic hydrolysis. Similarly, Kontogianni et al. reported and confirmed the efficacy of alkaline pretreatment in LCB waste, reporting that alkaline pretreatment of wheat straw with

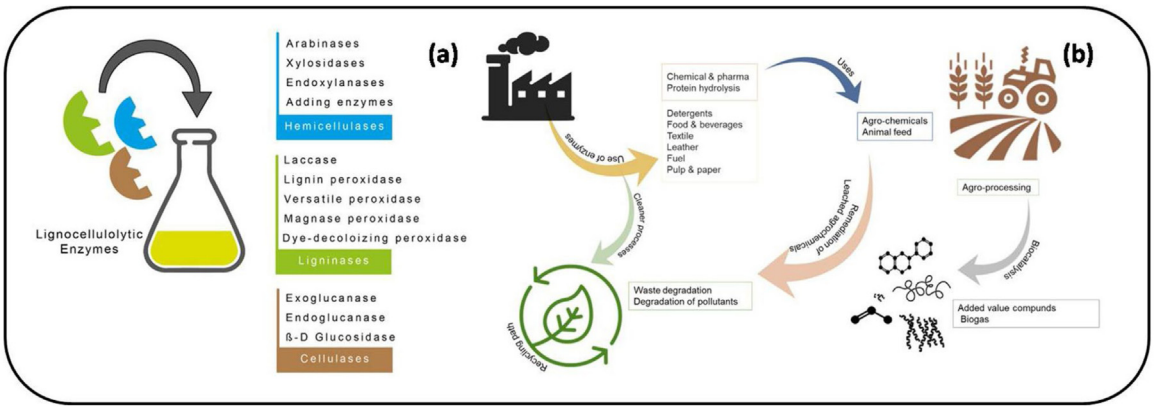

Fig. 2. Classification of lignocellulolytic enzymes (a) [adapted with permission from Saldarriaga-Hernández et al., 2020], Application of lignocellulolytic enzymes (b) [adapted with permission from Saldarriaga-Hernández et al., 2020].

10% H_2O_2 and 2.0% NaOH removed lignin by 89.60% up to and 84.86%, respectively (Kontogianni et al., 2019). Despite the fact that alkaline pretreatment has emerged as one of the most effective and significant methods, the amount of alkali required to pretreat the LCB prior to enzymatic hydrolysis raises the overall cost of the biofuels production process. As a result, an alternative pretreatment agent or method is still required to stabilize the cost of biomass-based biofuel production, where the cost of the hydrolytic enzymes is always expected to be lower.

3. Production of cellulose deconstructive enzymes using LCB waste

The cellulose de-constructive enzymes are a group of enzymes commonly known as cellulases. Cellulases are prime industrial enzymes that are specifically required in the production of biomass-based biofuels. There are three major sub-enzyme groups that, when combined, form cellulolytic enzyme complexes: endoglucanases (EG), also known as carboxymethyl cellulases; exoglucanases, such as cellodextrinase; cellobiohydrolase (CBH); and β-glucosidases (BGL). In general, the classification of cellulase enzymes is based on the function of cellulose depolymerization stages. For example, EG hydrolyzes glycosidic bonds that are present in the amorphous area of cellulose and produces oligomers after breaking the polymeric form. Exoglucanase, on the other hand, hydrolyzes the β-1,4-glycosidic bonds found between the oligomers at the reducing and non-reducing ends, resulting in cellobiose, which is then broken down into glucose by β-glucosidases (Behera et al., 2017; Efrinalia et al., 2022; Knott et al., 2014). The synergic action of all three enzyme components is required to function together to digest the polymeric structure of cellulose into monomeric sugars. Due to its high hydrolytic efficiency, cellulase plays a key role in the biofuel production process from LCB waste [Fig. 2] (Vasić et al., 2021; Zafar et al., 2022). Additionally, due to other industrial applications, demands for cellulases are always on the higher side on the global industrial market. Huge demand and production using commercial substrates make cellulase an expansive enzyme. Therefore, the development of low-cost cellulase using any inexpensive substrate is one of the prime focus areas of today's research. Production of these cellulases using LCB waste is the most sustainable and promising approach to bringing down the cost of the cellulases (Ravindran and Jaiswal, 2016; Sharma et al., 2016). Based on the microbiology of the microbial species involved, the production type and mechanism are different for cellulase in different microbial species. Cellulases are extracellular enzymes that are released as free molecules in an external microbial medium, while the same enzymes produced by aerobic bacteria are released and linked to the cell surface in the form of protein complex molecules called cellulosome. Several LCB wastes, such as rice straw, sugarcane bagasse, wheat straw, etc., are very well documented for potential cellulase production, and as per the studies, fungi are recognized as the better cellulase producers than bacteria. *Trichoderma* and *Aspergillus*, for example, are two potential reported fungi for cellulase production. Additionally, among fungal species, mycelium-producing fungal species are categorized as the most prominent group of cellulase-producing microorganisms (Delabona et al., 2020). Cellulase production using the low-cost method is focused on LCB waste utilizations, and it is also dependent on the fermentation pathway used, which is typically performed *via* solid-state fermentation (SSF) or submerged fermentation (SmF). Cellulase production using LCB wastes under SSF is regarded as the more viable method due to the maximum utilizations of solid substrate in the fermentation medium. However, types of LCB substrate, amount of lignin, microbial type, and overall bioprocessing development for maximizing enzyme production are the most challenging tasks, along with overcoming microbial deficiency to produce a complete cellulase enzyme system (Derntl et al., 2017; Siqueira et al., 2020; Wonoputri et al., 2018). As a result, researchers are investigating various horizons by implementing and establishing various strategies to achieve maximum cellulase production at a low cost (Han et al., 2020; Siqueira et al., 2020).

Cellulase is the subject of a huge number of research projects that aim to improve its hydrolytic performance. For example, in a recent study, Santos et al. used sequential fermentation (SF) of tree leaves collected as waste from urban

areas and applied it for endoglucanse (CMCase) and exoglucanase (FPase) production (Santos et al., 2022). Under solid-state fermentation and optimum conditions, 413.49 U/L CMCase as well as 230.68 U/L FPase could be produced at pH 5.5 and 75% moisture content. The main findings and conclusion of the study to produce enhanced cellulase were well-favored optimum substrate and bioprocess parameters. The selection of unique LCB in the form of tree leaves, which contain 20.36% cellulose and actively participated in all fermentation modes, was the key result of this study. In a study performed by Xiang et al. the maximum FPase activity of 19.85 IU/mL could be recorded under a continuous feeding system while using LCB waste *Miscanthus lutarioriparius* and the fungal species *Trichoderma reesei* RUT C30 (Xiang et al., 2021). *Miscanthus lutarioriparius* as a LCB has frequent ecological adaptability, higher growth, and stress tolerance, such as salt tolerance, and continuous feeding of the biomass gained better cellulase production as well as activity, as confirmed by the authors. The authors also referred to this study as low-cost due to the use of a potential fungal strain, and LCB following continuous feed in fermentation medium was probable due to higher cellulase and a reduced bioprocess. Further, Baskaran and Krishnan have isolated a novel fungal strain of Tricdetma species, identified as *T. gamsii,* which showed the highest cellulase production performance in 72 h at 28 °C in acidic fermentative conditions (Baskaran and Krishnan, 2020). In the study, FPase activity of 2.6 U/mL and β-glucosidase activity of 2.1 U/mL were measured using microcrystalline cellulose at a concentration of 13.7 g/L with the addition of mineral salts. The focused conclusion of the study was based on novel microbial strain identification to improve enzyme production. In the study of Karuppiah et al. co-cultivation strategies have been adapted by the investigators using the microbial strains *T. asperellum* GDFS1009 and *B. amyloliquefaciens* 1841 to enhance the cellulolytic enzyme production using substrates containing molasses, corn meal, and rice bran (Karuppiah et al., 2022). Mutual interaction of cocultured microorganisms and multi-substrate utilization are the key approaches of the study to improve enzyme production. This study also suggested that while trying the enhancing strategy of enzyme production, the selecting factors are potential in their individual forms, either in the case of a potential cellulase-producing strain or cellulose-rich feedstock. Following the same pattern, Moran-Aguilar et al. screened and evaluated three species of Aspergillus for cellulase production under the SSF using sugarcane bagasse as well as brewery spent grain (BSG) utilized as substrate after three pretreatments: alkaline, boiling water, and autoclave (Moran-Aguilar et al., 2021). The highest cellulase activity of 6.23 U/gds was recorded in the fungal strain *A. niger* CECT 2700 using BSG. The above findings highlighted the importance of low-cost substrate and microbial efficiency. In the study of Singhal et al. LCB wheat straw was used for cellulolytic enzyme (CMCase) production using *Aspergillus Flavus* (Singhal et al., 2022). The investigation was based on emphasizing the significance of bioprocess parameter optimizations, and thus, nitrogen source, inoculum load, and duration of enzyme production have been taken as variables. The highest 13.89 U/gds cellulase concentration was measured after 12 days of fermentation with a yeast extract (0.25%) and an inoculum of 0.625%. Moreover, the study was also focused on optimization model system suitability and evaluated response surface methodology-box behnken design (RSM-BBD)—and machine learning (ML) models. Further, the ML process was recorded to deliver better results than RSM and BBD. Thus, it was concluded by the authors that cost economy is also dependent on bioprocess parameters, model selection, and other bioprocess parameters (Singhal et al., 2022). Further, Intasit et al. (2021) studied synergic cellulase production and their activities using palm waste employed as substrate under the co-cultivation mode, using the fungal strains *Aspergillus tubingensis* TSIP9 and *Trichoderma reesei* QM 9414 (Intasit et al., 2021). The highest cellulases, 374.8 IU/g, and beta-glucosidase, 161.87 IU/g, were recorded using palm waste under the sequential SMF and SSF. This study also supported microbial cocultivation and LCB application as among the most promising approaches towards cellulase production enhancement. Noguchi et al. used random mutagenesis to improve cellulase production in *Trichoderma reesei* T1281, producing the highest FPase of 15.6 U/mL and the highest β-glucosidase of 53.8 U/mL on lactose inducer (Noguchi et al., 2021). Since *Trichoderma reesei* is a commercial cellulase producer, high FPase but low β-glucosidase levels have been documented, while random mutation and lactose inducer could be used to improve β-glucosidase production. In addition to this series, Andriani et al. used the same pattern to study thirteen Indonesian sorghum accessions with different lignin compositions and the fungus *Trametes hirsuta* AA-017 for sequential enzyme production (Andriani et al., 2020). Fairly high enzyme activities 25.7×10^3 laccase, 540 U/L cellulase and 670 U/L xylanase were recorded in this study. The authors said that the study's conclusion was that fungi behave selectively on certain types of biomass, interact with each other, and make sequential multicomponent enzymes for different uses. Furthermore, Zhang et al. reported significant improvements in fungal cellulase production via Trvib-1 gene overexpression in *Trichoderma reesei* Rut-C30 (Zhang et al., 2018). The cellulase and protein production were significantly higher by 200% and 219%, respectively, than the parent strain, which could be an opportunistic approach to enhancing cellulase production under optimized bioprocess parameters. Though several approaches have been well established, a series of continuous efforts to improve the cellulolytic enzyme production and advanced strategies are needed to enhance the functional efficiency and stability of enzymes to further improve the final hydrolytic efficiency of the cellulase enzymes for the highly efficient bioconversion process of LCB.

4. Biofuels from LCB waste

Biotransformation of LCB wastes into biorefinery products plays an important role in achieving zero carbon neutrality and also in the circular economy. Production of cellulase enzymes using LCB waste and use of these enzymes in the hydrolysis of LCB waste to produce fermentable sugars for biofuel production is one of the most sustainable valorizations of these wastes into value-added products following environmentally friendly strategies (Mohd Azhar et al., 2017).

Effective conversion of LCB waste into biofuels is highly dependent on various factors, such as types of substrate, functional efficiency of enzymes, and fermentative microbial communities that produce sugars for the efficient production of biofuels. Researchers are now using engineered microorganisms to make cellulases and break down biomass at the same time. One approach that uses a single microbe to make both cellulases and biofuels is called "consolidated bioprocessing" (CBP). In this process, a single microbe makes both cellulases and biofuel, but it is quite challenging to get good results (Cunha et al., 2020; Thapa et al., 2019). Another innovative approach may involve utilizations of engineered fungal species to produce different cellulases and xylanases and confer cellulose and hemicellulose. Furthermore, commercial cellulose can be used as a substrate to produce cellulase, which is a promising method for screening the microbial efficiency to produce cellulolytic enzymes (da Silva et al., 2018). Nevertheless, this is not always a useful method due to the high cost of the commercial substrate and considering the complex enzymatic hydrolysis process involved in the case of LCB. Furthermore, many studies have shown that cellulase produced by a single microorganism behaves differently on different substrates and even produces different isoforms of the same cellulase (Abdullah et al., 2018). Additionally, one of the biggest advantages of using commercial cellulose for cellulase production is that it will screen the highest cellulase producing microbes, which can be further grown on LCB substrate and biomass enzymatic hydrolysis. Use of the commercially stored enzymes may also hamper the accuracy of the final results of sugar and biofuel output, as has been confirmed by numerous research studies. Additionally, it is also concluded from many studies that the blend of different cellulases can offer better performance than single cellulase system (da Silva et al., 2022; Tushar and Dutta, 2020). Along with cellulase, other factors are also affected the enzymatic hydrolysis of the LCB waste, for instance, two-stage micro-reactor system supplemented with cellulose de-constructive enzymes was implemented for enzymatic hydrolysis of wheat straw as potential LCB waste. During the two-stage microreactor approach, the porosity and surface area of the wheat biomass were enhanced, which improved the saccharification rate. Despite the fact that the reaction was stopped after 36 h, the sugars obtained were 3.0 times higher than the control (Xia et al., 2022). Buragohain et al. conducted anaerobic digestion of three LCB wastes, namely duckweed, switch grass, and rice straw, in 1 L of reactor along with cattle dung codigestion (Buragohain et al., 2021). The results showed average daily biogas production of 0.36 m^3/kg-VS in the case of rice straw and cattle dung, while 0.34 m^3/kg-VS and 0.32 m^3/kg-VS have been obtained for switch grass and duckweed, respectively, under mesophilic conditions of 28–32 °C. The differences obtained in all three biomasses were based on the cellulose content of the biomass, which was responsible for the effective conversion of waste into biogas. Vu et al. explain the same phenomenon, arguing that the cellulose content of LCB waste is the deciding factor for its maximum value (Vu et al., 2020).

Nowadays, production of bioethanol from starch is a well-established technology in the commercial biofuels market, while ethanol from LCB waste is under pilot-scale demonstration. NREL (USA), Iogen Corporation (Canada), as well as ETEK (Sweden), are now in the process of producing pilot-scale ethanol annually from a few hundred to a few thousand liters using LCB waste as feedstock. Based on fermentation biology, the maximum theoretically achieved yield of hexoses (C_6) and pentoses (C_5) is reported around 0.511 kg ethanol as well as 0.489 kg CO_2 per kg sugar. Thus, the overall theoretical ethanol yield becomes 0.719 and 0.736 liters per kg of glucan and xylan, respectively, at 20 °C. Yeast, S. cerevisiae, can only ferment C6 sugar while other yeast as well as bacteria are currently under observation to use C5 sugars along with the trial of genetically engineered fungal species that can produce a high volume of cellulase, xylanase, and hemicellulose and efficiently convert cellulose and hemicellulose into fermentable sugars in a single titer (IEA, 2021). In a study by Mattam et al. a new yeast strain was identified as *Candida tropicalis* and expressed cellulase and hemicellulase enzymes over the different temperature ranges of 32 and 42 °C for the conversion of xylitol and ethanol *via* sugar hydrolyzate (Mattam et al., 2016). Also, when wheat straw was used as a feedstock, 49 g/L of xylose turned into 15.8 g/L of xylitol, and 25.4 g/L of glucose turned into 7.3 g/L of bioethanol. Consolidated bioprocessing (CBP) is the approach proposed in this research study, which is based on the utilization of a single microorganism for all processes, starting with enzyme production following biomass hydrolysis and fermentative fuel production. One of the major benefits of the CBP approach is that it reduces the cost of microorganism growth and multiple sub-culturing on different media, as well as maximizing the valorization of LCB, which can be used as a substrate for both enzyme and biofuel production. In a very recent investigation by Monir et al. integrated bioethanol production from hybrid gasification and syngas fermentation processes has been recorded as being highest from forest waste-based LCB (Monir et al., 2022). In the study's details, different LCB waste, such as empty fruit bunches (EFB) of palm oil and coconut shell, were also used, along with two fermentative microorganisms known as bacteria and yeast. The highest bioethanol concentration of 15.31 mmol/L was obtained in the case of forest waste-based syngas fermentation *via* yeast, while the lowest bioethanol concentration of 14.23 mmol/L was obtained in the case of EFB-based syngas fermentation *via* bacteria (Monir et al., 2022). The integrated method that could produce both syngas and bioethanol using LCB waste was the highlight of the study. In addition, the potential of different LCB wastes has been tested based on the microbial type variations and their efficiency. The study of Saini et al. focused on the performance of a microbial strain in hydrolyzing substrate as well as biofuel production (Saini et al., 2022). The study reported *Ganoderm alucidum* as a potential basidiomycetous fungus to produce laccase, xylanase, and cellulases and presented effective pretreatment of switch grass LCB *via* production of 510 U/mL of laccase, which gave 22.47 fold more sugar than the control in the biomass conversion process and showed a bioethanol yield of 1.96 g/L. This study concluded that laccase enzyme plays a critical and promising role in the economical biomass-based biofuels production process by lowering the cost of the pretreatment agent. In one of the other studies by Ziaei-Rad et al. a low-cost ionic liquid pretreatment (ILP) strategy was applied on wheat straw to produce bioethanol, and maximum production and yield of 43.1

g/L as well as 84.34% of bioethanol were achieved in 48 h of fermentation (Ziaei-Rad et al., 2021). As the key conclusive point, the ILP process was quite effective at achieving a high level of saccharification and released a glucose yield of 87.19% from 3 h of pretreated wheat straw biomass. Likewise, in another study reported by Sadhukhan et al. the significance of the combined co-fermentation of cellulose and hemicellulose for bioethanol production has been recommended to bring down the overall cost of the bioethanol (Sadhukhan et al., 2019). Patel Maulik et al. studied alkali-pretreated/steam-exploded wheat straw for 72 h to produce bioethanol, and the maximum peak ethanol yield was 0.46 g/g and 0.43 g/g of cellulose (Patel et al., 2020).

In addition to bioethanol, potential biohydrogen production from LCB waste has been well documented. Different pretreatment strategies of LCB wheat straw were used in the study by Zhu et al. to improve the saccharification and biohydrogen processes (Zhu et al., 2022). Lyophilization, hydrothermal, ultrasound, and dilute alkali post-cooking pretreatment methods have been applied, which effectively removed lignin and hemicellulose from wheat straw. By using ultrasonic and diluted alkali boiling on wheat straw, a reducing sugar of 13.18 g/L could be produced. In addition, a sequential biohydrogen yield of 133.6 mL/g total solids (TS) was recorded. Further, in the study of Katakojwala and Venkata Mohan, pretreatment of sugarcane bagasse (SCB) released cellulose (0.34 ± 0.02 g/g) and nanocrystalline cellulose (0.15 ± 0.02 g/g SCB) *via* the depolymerization process, and 0.15 L/g COD_R of H_2 was recorded (Katakojwala and Venkata Mohan, 2022). The key finding of this research investigation was a multi-product from the SCB's biorefinery, in which lignin and nanocellulose extraction were important, as well as biohydrogen production. Likewise, the significance of pretreatment and mixed culture-based H_2 production was studied by Medina-Morales et al. (2021). Acid pretreatment was able to produce higher sugars, while mixed culture could hydrolyze pretreated corncob which produced a maximum of 575 mL of H_2at 35 °C and pH 5.5. The study by Shanmugam et al. reported the pretreatment of sweet sorghum stover as a LCB waste through the laccase enzyme, which was obtained from the fungus *Trichoderma asperellum* and immobilized on Fe_3O_4@SiO_2-chitosan for biohydrogen production enhancement, and the H_2 production rates of 2.8 mol H_2/mol reducing sugar and 25 L H_2/L-d, respectively, were recorded (Shanmugam et al., 2020). The findings reported in this study suggested that the immobilized enzyme on nanomaterials can be reused in a number of reactions, which reduces the cost of the enzyme's utilization in multiple reactions. Similarly, three types of dehydrogenase enzymes were immobilized on mesoporous silica SBA-15 to improve enzyme stability in the bioconversion reaction of birch wood (Bachosz et al., 2022). The enzymes were investigated for 10 successive catalytic cycles as well as the storage time up to 10 days at 4 °C, and co-immobilized enzymes showed >70% catalytic efficiency. Like biohydrogen, biodiesel production from LCB waste has also been reported; for example, in the study of Vasaki et al. SCB substrate has been used for biodiesel production using catalyst K_2CO_3 for transesterification, and 80% of the biodiesel was achieved using pretreated biomass hydrolyzate (Vasaki et al., 2022). Pretreatment of SCB was performed *via* acid, alkaline, and ultrasonication, and 16.39 g/L of biomass concentration could be recorded using SCB hydrolyzate, whereas pretreatment of LCB was the core strategy of this study. In the observation of Patel Alok et al. *Cassia fistula L.* (CAE) as a LCB waste has been used for triglyceride production by the yeast *Rhodosporidium kratochvilovae*, which produced 53.18% (w/w) lipid when developed on CAE (Patel et al., 2015). Additionally, the microbial yeast strain, which is known as oleaginous yeast and can accumulate high quantity of triacylglycerides in their dry cell weight, was one of the key highlights of the study, along with the LCB type used for maximum valorization of this kind of LCB waste, which was fruit pulp from CAE. The impact of catalysts has also played a significant role in accelerating the biodiesel production process; for example, the significance of phyllosilicate-derived heterogeneous catalysts to accelerate the biodiesel production process has been discussed by Nawaz et al. (2022). In addition, mode of preparation and composition of the catalyst may also play a significant impact to influence the production process of biofuels [Fig. 3].

Also, substrate type, pretreatment methods, microbial strains, suitable bioprocesses, and nanocatalysts are important new factors that help improve the different types of LCB waste-based biofuel production processes. This fact has become clear from the above discussion and the many research studies. More in-depth and rigorous research analysis are required to further improve this process and related technology for mass-scale production while keeping its economic expenses low and making the entire process green to achieve carbon neutrality. Additionally, in recent studies, researchers have also focused on the combination of different LCBs to facilitate advent bioprocessing and thereby produce biofuels effectively. Furthermore, emphasis is being placed on advancing pretreatment strategies, either by using a biological or a combination of chemical pretreatment method that may consume less chemical reagent and thus make the overall process more practical. In addition, as per the recently reported research studies, emphasis must be given to the immobilization techniques of hydrolytic enzymes and whole cell microorganisms, along with recycling the used enzymes for more frequent uses.

5. Existing limitations and future directions

One of the major existing limitations currently faced by industries involved in the biomass-based biofuels production technology is the cost of enzyme production. Though broad applications and feasible production exist, the manufacturing of cellulolytic enzymes and their implementation to produce biofuels using LCB wastes appear very promising, but a number of technical roadblocks still need to be addressed, and these essentially require sustainable solutions. Unavailability of suitable cellulosic substrate, lack of optimization of bioprocess parameters, and inefficient and potential enzyme-producing microbial strains are the biggest constraints in the pathway of effective valorization of these

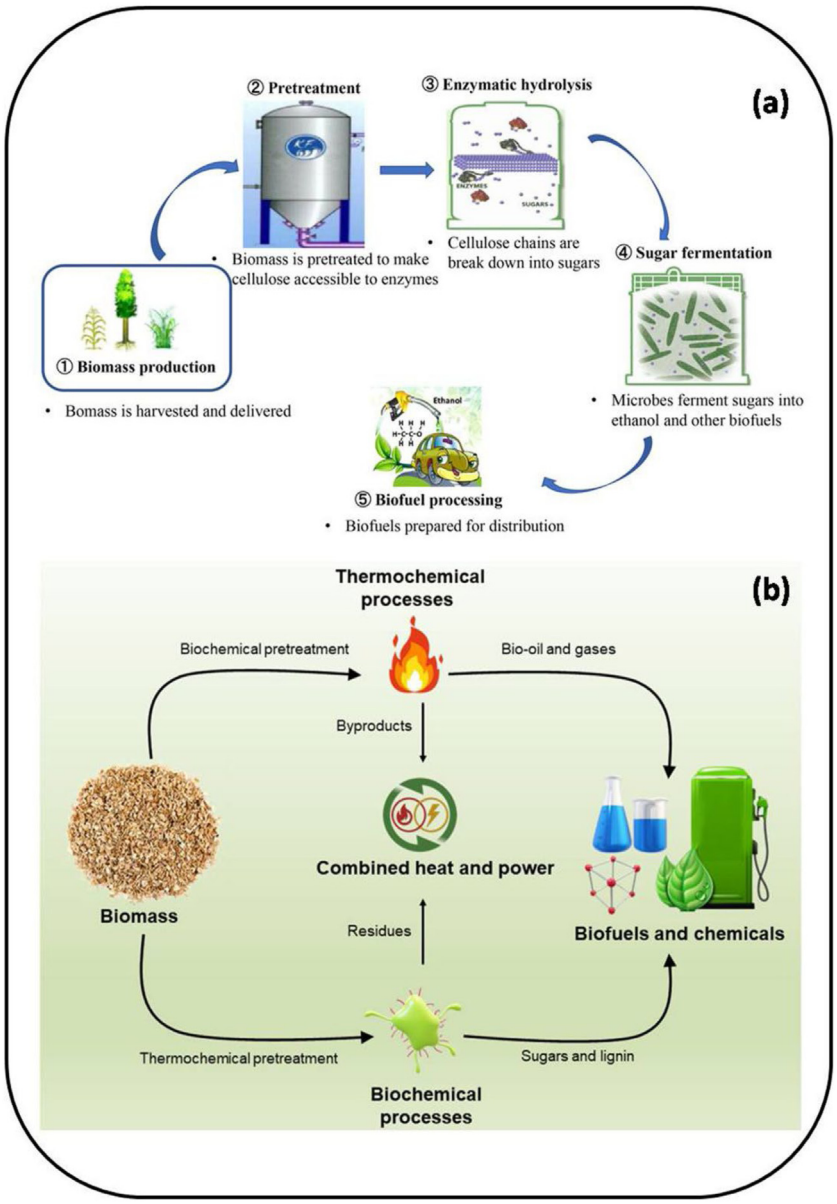

Fig. 3. Production process of cellulosic ethanol (a) [adapted with permission from Zhang et al., 2021], Integration of hydrothermal and biochemical routes in biomass utilization from a circular economy approach (b) [adapted from Osman et al., 2021 Open access CCBY 4.0]. . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

enzymes and biofuels applications. Thus, in the future, applications of genetics, genomics, proteomics, and metabolomics, as well as metabolic pathways and the related biotechnology engineering can make significant contributions to the development of cost-effective enzymes and biofuels (Bilal et al., 2018; Rathore et al., 2022). Additionally, using methods like metabolic engineering, heterologous gene expression of enzymes, and mutagenesis may increase the production of the enzyme systems as well as their catalytic effectiveness (Paul et al., 2021). Furthermore, genetic modification based on clustered regularly interspaced short palindromic repeats (CRISPR) in cellulase-producing microorganisms has been targeted recently to improve the catalytic efficiency of the cellulase enzymes in the bioconversion reaction of cellulosic substrate. The production of cellulases from yeast, bacteria, and fungi as well as their methods for enhanced catalytic capabilities may also be the focus of research (Imran et al., 2016; Naher et al., 2021; Okal et al., 2020; Sohail et al., 2022). Moreover, bioinformatics-based tools will also be helpful to explore the structural biology of the enzymes to enhance their catalytic activity as well as their potential based on the type of selected microorganism. Furthermore, the combination of genetic algorithms or machine learning approach with structural bioinformatics can be used to create

artificial design and tools for structure motifs in order to improve enzymatic efficiency *via* engineered protein, cloning, and protein purifications, as well as its overexpression in selected microorganisms. Further, microbiome analysis of selected samples and their storage may enhance the potential of cellulolytic enzymes *via* group of microbial screening and the construction of a genomic library (Balla et al., 2022; Singh et al., 2022). Aside from the total cellulosic content and substrate properties, the performance of the enzyme production will be influenced by microbial population and substrate interaction. Total cellulose content and enzyme production are directly proportional, while substrate surface area also plays an important role in improving enzyme production. For example, a rough surface of the substrate promotes fungal cellulase production because the microbe's mycelium easily covers the surface of the substrate and thus moves towards fast growth by spreading mycelium network, whereas a smooth surface of the substrate does not support this process (Paul et al., 2021; Peciulyte et al., 2014; Pihlajaniemi et al., 2016).

Another concern for economic cellulase and biofuels production at mass scale towards carbon neutrality is low functional stability of the enzymes and less tolerance over higher temperatures relative to the optimum value. Catalysts, in this view, play an important role in enhancing the functional stability of enzymes, and the performances of nanomaterial-based nanocatalysts are noteworthy to mention here and have been reported in numerous recent studies. Nanomaterials, when they function as catalysts, enhance enzyme production by improving the microbial metabolism and functional stability of the enzymes for better performance. In terms of the functional stability of enzymes, nanocatalysts improve the working temperature and pH stability of the enzymes to values that are more or relatively higher than their optimum values. Additionally, at very low concentrations, nanomaterials can influence the functional stability of microorganisms and enzymes either *via* more of a sorption or surface reaction or immobilization (Arsalan and Younus, 2018; Huang et al., 2020). The only thing that can hinder making this process more economical is the cost involved in the synthesis of nanomaterials and its toxic synthesis protocol. Therefore, more in-depth and rigorous research studies are required on the preparation of approaches to nanomaterials via green routes. Furthermore, significant efforts should be made to develop innovative synthesis methods and minimize their toxic effects during the preparation of nanomaterials in order to make the process more cost-effective and environmentally friendly toward carbon neutrality (Singhvi et al., 2022).

6. Conclusion

Cellulolytic enzymes and biofuel production are the most sustainable transformations of LCB waste toward the development of cost-effective and environmentally friendly carbon neutrality. However, owing to complex structural properties and diverse characteristics of LCB waste, practical low-cost transformation of these enzymes and their applications in biofuels production technology is the most difficult. Therefore, a thorough and critical analysis of LCB substrate is required before making substantial efforts and improvements to design an effective and affordable enzyme system. Also, the sound bioprocessing is required to produce a range of biofuels to further achieve more sustainable and economic processing toward valorizations and carbon neutrality for the industrial production prospective of these bioproducts is highly demanding. In this scenario, applications of advanced microbial engineering tools are expected to contribute to and accelerate the entire concept for the sustainable development of this area.

CRediT authorship contribution statement

Neha Srivastava: Conceptulization, Writing original draft. **Rajeev Singh:** Writing original draft. **Pardeep Singh:** Review and editing. **Irfan Ahmad:** Review and editing. **Ravindra Pratap Singh:** Review and editing. **Ashutosh Kumar Rai:** Review and editing. **Mohammed Asiri:** Review and editing. **Vijai Kumar Gupta:** Conceptulization, Review and editing original draft.

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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