REVIEW

Current progress on lignocellulosic bioethanol including a technological and economical perspective

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Abstract

Growing interest in lignocellulosic bioethanol stems from the European Union's renewable energy directive, targeting a global bioethanol output of 130 billion L to achieve a minimum threshold of 42.5%. Despite industrialization challenges, recent advancements, especially in crucial stages like pretreatment, hydrolysis, and fermentation, are transforming the landscape. This review delves into the progress of bioethanol production, addressing technological, environmental, and economic hurdles. Innovations such as deep eutectic solvent pretreatment and mechanocatalysis, offering advantages like 30% and 100% solid loading, respectively, surpass traditional techniques and enzymatic hydrolysis in yielding better results. Improved pretreatment methods, enzyme exploration, saccharification techniques, genetic engineering, and integrated biorefineries contribute to overall economic viability. Ongoing research involves techno-economic analysis for cost-effective strategies, aiming to enhance the competitiveness of lignocellulosic bioethanol production. Scrutinizing the feasibility of these innovative approaches not only highlights their potential to overcome existing shortcomings but also envisions a more attractive future for industrial bioethanol production. Embracing these advancements could pave the way for a vibrant and sustainable bioethanol industry.

Abbreviations
LCB   Lignocellulosic biomass
1G   First generation
2G   Second generation

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| BDES	Binary deep eutectic solvent
ChCl	Choline chloride
CMC	Conventional mechanocatalysis
DAP	Dilute acid pretreatment
DAMC	Direct acid impregnation before ball milling
DES	Deep eutectic solvents
NADES	Natural deep eutectic solvent
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HMF	5-Hydroxymethylfurfural
MM	Million
OP	One-pot system
PSSF	Presaccharification and simultaneous saccharification and fermentation
SE	Steam explosion
SHF	Separate hydrolysis and fermentation
SLS	Solid liquid separation
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous hydrolysis and fermentation
TDES	Ternary deep eutectic solvent
TEA	Techno-economic analysis
TSY	Total sugar yield
WSP	Water-soluble products

1 Introduction

Global warming, driven by increased greenhouse gas emissions from fossil energy sources, has prompted research into renewable and carbon–neutral alternatives like lignocellulosic biomass (LCB). This change is needed to respect the global warming limits determined in the Paris COP 21 agreement (Delmas et al., 2022). LCB is a versatile resource for sustainable biofuels, including biogas, biodiesel, and bioethanol. These biofuels contribute minimally to greenhouse gas emissions and foster agricultural development (Sondhi et al., 2020). Bioethanol, derived from the hydrolysis and fermentation of LCB like agricultural and forestry residues, holds significance as a biofuel and a precursor for hydrogen production, serving as a versatile energy source.

Currently, industrial bioethanol production relies on first-generation (1G) feedstocks like corn, sugarcane and wheat. In 2022, the United States, as a major producer, generated 58.1×10^6 m^3 of bioethanol; Brazil and the European Union produced 28.4 and 5.5×10^6 m^3, respectively (Sönnichsen, 2024). In the EU, 7% of the total cereal harvest (12.2 million metric tons) was required to produce 5.5×10^6 m^3 of bioethanol in 2019 (Flach et al., 2019). Typically, the cost of the 1G feedstock accounts for 40 to 70% of the total cost of the production of ethanol (Calderon & Arantes, 2019). For instance, the cost of corn and sugarcane were \$186/ton and \$61/ton while the prices for corn stover and sugarcane bagasse were estimated as \$59/ton and \$36/ton (Calderon & Arantes, 2019). The debate over food security versus energy prompted the need to replace 1G feedstocks with second-generation (2G) alternatives, which are cheaper and abundant. 2G feedstocks include agricultural residues, forestry residues, and organic fractions.

Unlike 1G, 2G feedstocks have complex structures requiring intensive pretreatment, increasing production costs (Silva Ortiz et al., 2020; Vasconcelos et al., 2020). For instance the minimum selling price for corn stover sourced sugar was estimated at \$587/ton which was about 2 times the reported price of sugar, \$283/ton (Calderon & Arantes, 2019). This limited the industrial development of bioethanol production from 2G feedstocks to ca. 50×10^3 m^3 in 2019 (Flach et al., 2019). Despite cost challenges, 2G feedstocks offer advantages in CO_2 emissions and energy efficiency (Silva Ortiz et al., 2020). Wood and agricultural residues are considered the most economically and environmentally competitive 2G feedstocks among lignocellulosic biomass, owing to their advantage to high hemicellulose content and optimal lignin levels for energy efficiency. The minimum selling price for alder wood, pine wood, wheat straw and corn stover sourced bioethanol were \$2.07, \$2.13, \$2.05 and \$2.17/US gal while elephant grass and guinea grass were \$2.45 and \$2.54/US gal (Sadhukhan et al., 2019).

The 2G feedstocks are formed by three natural polymers, cellulose (30–60%), hemicellulose (20–40%) and lignin (15–25%), which constitute lignocellulosic biomass (LCB, Fig. 1). Theoretically, from one ton each of glucan from cellulose and xylan from hemicellulose, 651.1 L and 666.2 L of bioethanol could be produced through fermentation, respectively (Calderon & Arantes, 2019). Lignin acts as a shield against both chemical and biological attacks of the cellulose and hemicellulose portions. Pretreatments to remove lignin from LCB and make cellulose and hemicellulose fermentable are an essential part of the process to obtain bioethanol from 2G feedstocks. Chemicals such as acids, alkalis, organic solvents, oxidising agents, ionic liquids and deep eutectic solvents have been used for this purpose. However, pretreatment can increase the cost of bioethanol up to an additional 40%. Major issues when designing a pretreatment process are the generation of chemical waste and the requirements of primary energy per unit mass of processed biomass. Current recognition of green and clean processes makes it paramount to consider the pretreatment not only from the aspect of cost but also by its environmental friendliness, recyclability of chemicals and overall minimization of the environmental impact.
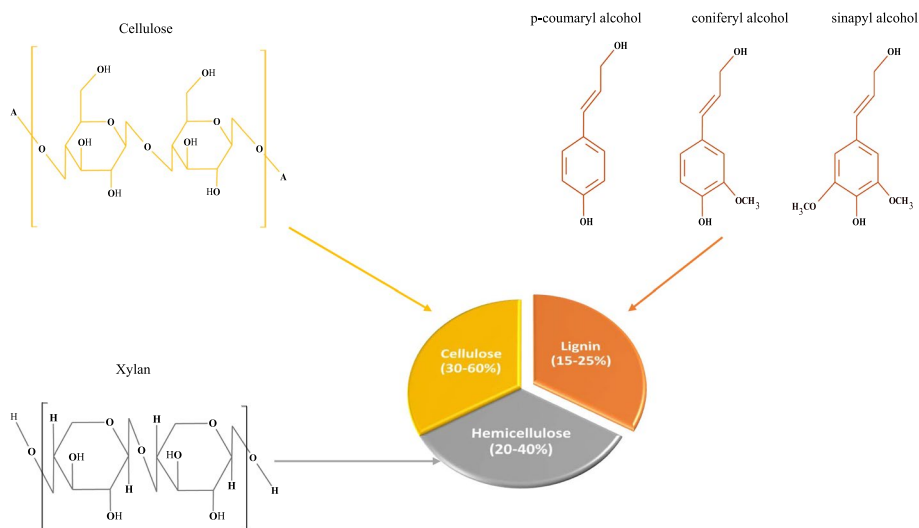
Fig. 1 Composition and structure of the main components of lignocellulosic biomass

Hydrolysis of the cellulose-rich pretreated substrates and fermentation of the glucose formed are the subsequent stages of the bioethanol production process. These two steps can either be performed consecutively through separate hydrolysis and fermentation (SHF), or by simultaneous hydrolysis and fermentation (SSF). The effectiveness and the cost of the enzymes and yeasts used in these steps are of significant concern in bioethanol production. For instance, even at a low cellulase dosage of 20 mg cellulase/g glucan, the cost of the enzyme amounts to 15.7% of the cost of the bioethanol produced from LCB (Chen et al., 2021a, 2021b). Although hydrolysis can lead to high yields of monosaccharides (glucose and xylose), *Saccharomyces cerevisiae* (*S. cerevisiae*), the yeast strain most commonly used is only effective in fermenting glucose while xylose is not converted to bioethanol. Since most of the enzymes and yeasts that are used are commercial strains, research to improve efficiency and reduce process cost has focused on the optimisation of the pretreatment strategy, process integration, yeast recycling, and in obtaining yeast strains capable of fermenting xylose (Bibi et al., 2023).

A study of the industrial status of 2G lignocellulose bioethanol plants around the world showed that although a lot of companies were constructed in the U.S, Germany, China, Brazil and Italy among others, the cost of bioethanol production with 2G biomass far exceeded that with 1G biomass resulting in most of them shutting down (Wang et al., 2022). The few companies that have survived until now have mainly being due to innovations in the field of pretreatment, hydrolysis or fermentation.

The present Renewable Energy Directive of the European Union (EU/2023/2413) aims to attain a minimum renewable energy threshold of 42.5%, aspiring to elevate it to 45% by the year 2030 (European Commission, 2024) This signifies nearly doubling the present proportion of renewable energy within the EU. Furthermore, with 85% of Europeans believing that EU should make substantial investments in renewable energy there is bound to be a rise in investments. This signifies that despite the cost involved in bioethanol production from 2G biomass, the process could see a boost in investment. Furthermore, bioethanol cost is directly linked to the ethanol concentration and yield meaning that lignocellulose bioethanol can become sustainable when the ethanol concentration exceeds 40 g/L (Chen & Fu, 2016). Bioethanol production from 2G lignocellulosic biomass can become more feasible with a reduced total cost in the forthcoming future by improving the pretreatment process, saccharification performance, enzyme price and fermentation efficiency (Carvalho & Ishikawa, 2019). There is therefore a need to take a second look at the bioethanol production technological process and determined its shortfalls, as well as means to remedy it to make it industrially and economically feasible.

To this end, this article reviews and summarizes the current challenges in the production of bioethanol from 2G lignocellulose biomass, focusing on pretreatment, hydrolysis and fermentation stages. Current strategies to address those challenges and their implication on current ethanol production and techno-economic are highlighted and future trends in process configurations are discussed. It is envisioned that effectively incorporating these strategies would further empower and promote the use of industrial production of bioethanol from LCB.

2 Challenges in the bioethanol production process

2.1 Pretreatment

Pretreatment techniques are classified as chemical, physico-chemical, physical or biological. Each type of pretreatment has its own merits and demerits (Table 1). An efficient pretreatment technique reduces the lignin content thereby preventing enzyme attachment to lignin during hydrolysis improving saccharification efficiency (Preethi et al., 2021). The individual pretreatment techniques have been extensively reviewed in literature (Gandam et al., 2022). Physical pretreatments focus on particle size reduction to increase surface area, reduce cellulose crystallinity and improve the accessibility to enzymes and microorganisms (Maroušek et al., 2012; Sitotaw et al., 2023). They have a high energy requirement, but do not form inhibitors and have a minimal effect on the removal of lignin and hemicellulose. Chemical pretreatments require less time than biological treatment and less energy than physical treatments (Li et al., 2022; Maroušek, 2013). They cause changes on the lignocellulosic structure that remove lignin or/and hemicellulose partially and develop porosity and accessibility to the cellulose fibres, but they generate inhibitors and produce chemical waste that requires of treatment and disposal (Ceaser & Chimphango, 2021). Physico-chemical pretreatments combine characteristics of the physical and the chemical treatments and inherit both their positive and negative qualities. Biological pretreatments are environmentally friendly, selective and do not form inhibitors of the fermentation microorganisms but require costly nutrients for microbes and extensive pretreatment time.

The particular advantages of different pretreatments have led to their integration into sequential or combined pretreatment techniques that enhance delignification efficiency but require less time and energy, and reduce the losses of sugars, the formation of inhibitors, the generation of waste, and ethanol cost. Combining fungi treatment with steam explosion (SE) reduced the time required in the biological pretreatment from 60 to 35 days (Taniguchi et al., 2010). A sequential combination of dilute acid pretreatment (DAP) and SE removed 75% xylose, 18% glucose and 22% lignin from olive stones but formed acetic acid and furfural from xylan as the main fermentation inhibitors (Padilla-Rascón et al., 2020). Interestingly, bacteria-enhanced DAP did not significantly affect the biomass composition as opposed to a simple DAP, however it improved biomass digestibility by 70% in comparison with a solo DAP (Yan et al., 2017). The authors showed that the bacteria attached itself to the lignin droplets formed on the biomass during pretreatment thereby not causing a decrease in lignin content. Nevertheless, this action of the bacteria promoted the porosity of the lignocellulose matrix while preventing lignin from hindering saccharification, thus improving the digestibility and the yield of glucose at similar biomass compositions.

Microwave pretreatment is another method that over the past decade has gained much interest, especially when used in combination with other pretreatments (Ocreto et al., 2021). The microwave irradiation increases the rate of chemical reactions, reducing treatment time whereas causing an explosion effect within the biomass structure thereby improving pretreatment efficiency (Table 1) (Amesho et al., 2022; Kumari & Singh, 2018). Microwave pretreatment was reported to perform better in improving biomass composition and sugar yields than both autoclave and hot plate heating (Gabhane et al., 2011; Shangdiar et al., 2023). Water, acids and alkalis are some of the chemicals that have been used in microwave pretreatment. Microwave-assisted water pretreatment was reported as an

Table 1 Advantages and disadvantages of pretreatment methods

Pretreatment method	Advantages	Disadvantages
Physical		
Grinding, chipping and milling	Reduction in particle size, reduction in crystallinity, no chemical usage, no inhibitor formation, improve enzymatic digestibility of pretreated residue	High capital cost, high energy consumption, does not remove any biopolymer from the biomass
Chemical		
Dilute acid	Improved digestibility, reduction in crystallinity, hemicellulose removal, lower reagent cost	Little effect on lignin, requirement for specialized equipment, acid neutralization required, inhibitor formation, detoxification required to remove inhibitors
Alkaline	Improved digestibility, lignin and hemicellulose removal, reduction in crystallinity, improved substrate porosity, no need for specialized equipment, low treatment temperature	High cost of reagents, longer treatment time, neutralization of slurry
Organic solvent	Easy substrate recovery, high lignin removal, high purity lignin obtained as co-product	High cost of organic solvent, formation of inhibitors, high energy demand, highly flammable and volatile, risk of explosion due to high pressures
Deep eutectic solvent	Ease of preparation, biodegradable, recyclable, reusable, less costly, operated at low temperatures, low energy demand, high lignin and hemicellulose removal, improved porosity, improved digestibility,	Highly viscous
Physico-chemical		
Liquid hot water	No chemical usage, low environmental impact, increased particle porosity, high lignin and hemicellulose removal, little inhibitor formation	High energy demand, high water usage, high capital investment
Steam explosion	Low chemical usage, low environmental impact, increased particle porosity, high hemicellulose removal	High energy demand, high capital cost, inhibitor formation at harsh condition
Biological	Low chemical usage, low environmental impact, very specific	Very long treatment time, requires specific treatment conditions and management of microbes
Combined pretreatment (using microwave)	Short reaction time, faster heat transfer, environmentally friendly, highly selective, little inhibitor formation	Poor distribution of microwave power, low penetration radiation, high energy demand, currently still being applied on a lab-scale

efficient method for obtaining polymeric and oligomeric xylan yields (51–66%) from bio-mass while efficiently increasing content and digestibility (74–78%) of cellulose (Mihiretu et al., 2017). Similarly, 38% hemicellulose and 74% cellulose were retained while 69% lignin was removed from wheat straw using microwave assisted NaOH pretreatment. This increased the saccharification efficiency by 79% while reducing the time taken to reach the highest saccharification efficiency from 12 to 8 days in comparison with untreated wheat straw (Tsegaye et al., 2019). Unlike the low inhibitor production when water and NaOH are used as the chemicals, the use of H_2SO_4 in a microwave-assisted pretreatment of maize distillery stillage was reported to produce enough inhibitors that detoxification was required before undergoing fermentation (Mikulski et al., 2019).

The major inhibitors produced during the pretreatment process are furfural, 5-hydroxymethylfurfural (HMF), phenols, and carboxylic acids such as acetic acid. The inhibitors produced depend on the pretreatment technique, the chemicals used and their toxicity, the pretreatment conditions and also the composition of biomass itself (Preethi et al., 2021). In acidic pretreatments, furfural, HMF, phenols and carboxylic acids are formed as inhibitors while in alkaline pretreatments phenols, carboxylic and acetic acids are formed (Beig et al., 2020). In SE, the partial removal of lignin and hemicellulose and the formation of inhibitors such as furfural and HMF makes it easier for the inhibitors to penetrate the biomass cell wall affecting enzymatic and microbial activities (Simangunsong et al., 2020). In hydrothermal or liquid hot water pretreatments, the elevated water temperature also causes the formation of furfural from the extracted hemicellulose (Paul & Dutta, 2018). Excessive delignification results in lignin depolymerisation into acids that can also serve as a source of inhibition (Alvira et al., 2010). Silica present in biomasses such as rice husks can also act as an inhibitor to cellulolytic enzymes after scaling mechanical equipment (Satlewal et al., 2018; Talukder et al., 2017).

Cost is a major consideration in the feasibility of industrialising any conversion process. Pretreatment alone can make up from 11 to 27% of the total cost of bioethanol production (Su et al., 2020). Energy and chemicals are the major contributors to the cost of pretreatment. A study conducted on a fermentable sugar production process by (Baral & Shah, 2017) showed that a biological pretreatment was over three times more expensive than DAP or SE, although it required 12-fold less energy due to the lower temperature required. This was due to a twofold increase in the required amount of feedstock and the much larger reactor volume that was needed due to the long pretreatment time (23 days) to achieve similar yields of fermentable sugars. A techno-economic analysis conducted on a 2000 t/day bioethanol production process showed that pretreatment cost was higher in DAP ($25.0 million (MM)) and alkaline ($23.0 MM) than in hot water ($4.5 MM), while the total capital cost was the lowest in alkaline pretreatment ($163.6 MM) than in DAP ($208.6 MM) and autohydrolysis ($200.9 MM) (Eggeman & Elander, 2005). Tao et al. (2013) assessed sugar production through various pretreatment techniques and the cost involved. DAP, alkaline, autohydrolysis and SO_2-SE gave sugar yields of 398, 427, 315 and 459 million kg/year for 2000 tons of biomass processed per day; however, the installed equipment cost for pretreatment increased in the order autohydrolysis pretreatment ($10 MM) < DAP ($23 MM) < SO_2 SE ($33 MM) < alkaline pretreatment ($86 MM). Alkaline pretreatment had the highest equipment cost because it was the only process that included a recycling step. Although the cost of the alkaline pretreatment reactor was lower than that of the more complex reactors needed for DAP and SO_2 SE, the longer residence time and number of reactors required contributed to the higher investment. The inclusion of a detoxification unit for treatments such as DAP and SO_2 SE could have further increased the final cost (Baral & Shah, 2017; Preethi et al., 2021).

In chemical pretreatment, the chemical requirements cost is normally high due to the inclusion of other expenses such as chemical storage and chemical transportation cost (Paudel et al., 2017). However, this expenditure can be offset by achieving high product yields (Preethi et al., 2021). Dilute acid pretreatment was reported to yield 0.27 t bioethanol/t biomass while liquid hot water and steam explosion yielded 0.19 and 0.15 t bioethanol/t biomass, respectively. As observed in the study by Eggeman and Elander (2005), the direct chemical pretreatment cost is over fivefold higher than with the use of autohydrolysis; there is, therefore, a need for recovery, recycle and reuse of the chemicals in order to reduce this expenditure (Baral & Shah, 2017).

2.2 Hydrolysis

The hydrolysis step in bioethanol production involves the conversion of cellulose and hemicellulose to glucose and xylose or mannose, respectively. Crystallinity makes cellulose harder to hydrolyse than hemicellulose. To hydrolyse cellulose successfully, acids (chemical hydrolysis) or enzymes (enzymatic hydrolysis or saccharification) can be used.

Chemical hydrolysis with dilute acids is preferred to concentrated acids due to less inhibitor formation, reduced equipment corrosion, and absence of acid recycling, which reduces investment. Dilute acid hydrolysis is conducted at 1–5 wt% acid for 3–120 min at 180–240 °C (Abo et al., 2019). Glucose yields of ca. 60% are typically achieved but hemicellulose is mostly degraded to furfural and humin substances. A two-step process was suggested with the first step focusing on xylan hydrolysis (140–160 °C) and the second step focusing on cellulose hydrolysis (160–180 °C). Such a system could increase the total sugar yield (TSY) to 80% with the production of less inhibitors (Hamelinck et al., 2005). Even if yields are improved with this two-step approach, the challenges of the chemical hydrolysis process still exist, although they are minimised substantially.

Enzymatic hydrolysis is the preferred form of hydrolysis due to the low temperature, near-neutral pH, reaction specificity, absence of inhibitor production and higher selectivity rates in comparison with chemical hydrolysis (Binod et al., 2011; Galbe & Zacchi, 2007). Enzymatic hydrolysis is a promising alternative to dilute acid but has two major challenges. One is the high cost of the enzymes, which account for ca. 16% of the bioethanol cost. The second is the long treatment time (72 h) needed to achieve maximum yield (El-Zawawy et al., 2011; Humbird et al., 2011; Yu et al., 2016). Most of the enzymes used in the process are commercial grade enzymes and the cost associated with their application brings the feasibility of this process into question. In the production of bioethanol from LCB, enzyme cost is one of the major bottlenecks (Binod et al., 2011). Due to this, most researchers have focused on the use of lower enzyme concentrations, using new cellulolytic cocktails or substitution of the enzymatic hydrolysis with other treatments to achieve similar or higher yields (Abo et al., 2019). Also, with enzymatic hydrolysis the solid loading is usually maintained < 5% to prevent technical problems such as ineffective mixing and mas and heat transfer defects that might impair the enzyme activities (Broda et al., 2022).

2.3 Fermentation

Fermentation is the biological conversion of glucose (and xylose) into alcohol by an enzyme secreted by a microorganism. In bioethanol production, the two microorganisms mostly used to convert glucose are *Saccharomyces cerevisiae* (*S. cerevisiae)* and *Zymomonas mobilis* since their tolerance to ethanol facilitates high conversion yields.

However, the susceptibility of *Zymomonas mobilis* to contamination makes *S. cerevisiae* the most used microorganism for bioethanol production. The wild strain of *S. cerevisiae* commonly used can only ferment hexose sugars (Eq. 1). A different microorganism is therefore needed to ferment xylose and pentose sugars in general (Eq. 2).

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \tag{1}$$

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \tag{2}$$
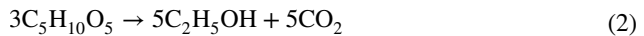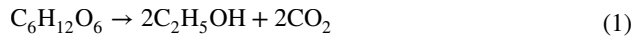
Equations 1 and 2 represent the formation of ethanol from glucose and xylose, respectively. Both equations show that given 1 g of either glucose or xylose, the stoichiometric maximum amount of ethanol that can be produced is 0.511 g. However, this yield is not obtained in reality due to the formation of other metabolic products such as acetate, lactate, glycerol, xylitol and hydrogen (Robak & Balcerek, 2018; Scully & Orlygsson, 2015).

The fermentation process may be performed separately from the hydrolysis process or simultaneously (Fig. 2). Separate hydrolysis and fermentation (SHF) is a sequential process involving hydrolysis and fermentation in two separate reactors. Usually, enzymatic hydrolysis is conducted at 45–50 °C to produce sugars after which they are fermented to alcohol at a lower temperature (30–37 °C). This configuration is faced with the challenge of the produced glucose inhibiting the activities of β-glucosidase and cellulase (Abo et al., 2019). Furthermore, the capital cost is increased due to the need for two reactors for the process and the time taken. However, this configuration allows for easier recycling of the fermentation microbes.

Simultaneous saccharification and fermentation (SSF) involves performing the hydrolysis and fermentation process in the same reactor at the same time. In this configuration, the sugars produced from the hydrolysis are immediately fermented by the microbes to ethanol, thereby reducing glucose inhibition and contamination (Fig. 2). The SSF has a lower capital cost and higher yield in comparison with SHF. The major challenge of SSF is the lower sugar yields due to the reduced temperatures (< 37 °C) required for the survival of the fermenting microorganisms, which in consequence reduces the yield of ethanol.

A comparison of SHF and SSF shows that the SSF configuration gave higher yield and ethanol productivity than SHF (Table 2). Fernandes et al. (2018) compared SSF and SHF for cardoon and rockrose pretreated with DAP; ethanol yield and productivity were higher in SSF, and the reaction time was shorter in SSF (24 h) than in SHF (72 h + 24 h). At the optimum conditions for a lignocellulose mixture, SSF required less cellulase (80 U/g) and longer time (30 h) and gave higher ethanol productivity (1.396 g/L/h) than SHF. The latter used 132.9 U/g of cellulose for 27.33 h to yield only 0.929 g/L/h of ethanol (Althuri & Banerjee, 2019). SSF (41.9 g/L) gave 1.64-folds higher ethanol yield than SHF (25.40 g/L). Other researchers have also observed a reduced ethanol yield with similar ethanol productivities from SSF in comparison with SHF and have stated that the lower temperature for the hydrolysis in the SSF was the main reason for the inferior yield (Guerrero et al., 2018; Thanapimmetha et al., 2019). The efficiency of the process was therefore affected by the lack of thermophilic or pentose-fermenting microbes. Therefore, although SSF has become more attractive, the process demands an improvement to improve ethanol yields.
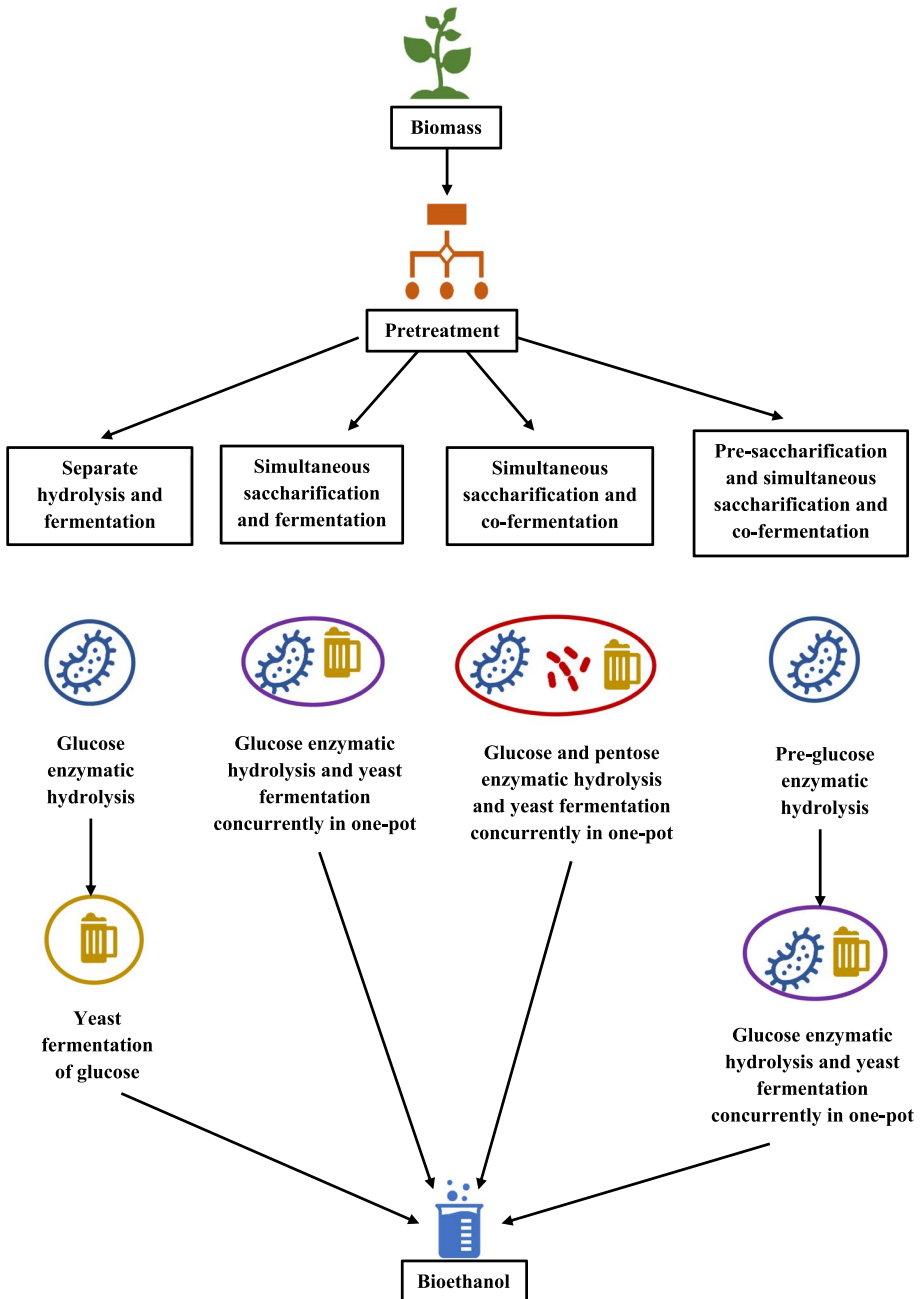
Fig. 2 The stages in the bioethanol production process for different production routes

Table 2 Ethanol yield and productivity from biomass pretreatment, hydrolysis and fermentation using *Saccharomyces cerevisiae*

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Rockrose	6.7% (w/w) sulphuric acid solution at 130 °C in an autoclave for 55 min	SHF—enzymatic hydrolysis at 150 rpm and 50 °C for 72 h with Celluclast 1.5L and Novozyme 188. Fermentation with *S. cerevisiae NCYC 1119* at 30 °C, 130 rpm for 24 h. SSF—enzymes and thermotolerant yeast, *S. cerevisiae PYCC 2613* at 42 °C for 24 h	SHF—4.7 SSF—5.2	SHF—0.20 SSF—0.22	Fernandes et al. (2018)
Cardoon			SHF—11.5 SSF—12.2	SHF—0.48 SSF—0.51	
Cardoon	Steam explosion for 1 min at 235 °C, 3.2 MPa. Then soaked for 24 h in distilled water, vacuum filtered and then treated for 15 min with 2% sodium hydroxide solution	SHF—enzymatic hydrolysis was conducted with Celluclast 1.5L and Novozyme 188 at 50 °C and 130 rpm for 72 h. Fermentation with *S. cerevisiae NCYC 1119* at 30 °C, 130 rpm for 24 h. SSF—it was conducted at 42 °C and 130 rpm for 72 h with the same medium preparation as for SHF experiments	SHF—18.5 SSF—18.7	SHF—0.77 SSF—0.78	Fernandes et al. (2015)

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Lignocellulosic feedstock mixture	Enzymatic delignification of 1 g of mixed substrate with 3.333 mL laccase (pH 7) at 30% (w/v) incubated at 35 °C for 6 h	SHF—enzymatic hydrolysis with cellulase and xylanase at 50 °C for 10 h. Fermentation with *S. cerevisiae* at 38 °C for 27.3 h. SSF—it was conducted by incubating *S. cerevisiae* together with cellulase and xylanase, and delignified lignocellulosic mixture feedstocks at 15% (w/v) final substrate concentration for 30 h at 37 °C	SHF—25.40 SSF—41.89	SHF—0.929 SSF—1.396	Althuri and Banerjee (2019)

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Banana rachis	Acid-catalyzed steam explosion with 1.5% H$_2$SO$_4$ (v/v) at 198 °C and 5 min	SHF—enzymatic hydrolysis at 50 °C during the first 24 h. Then, the bottles were transferred to an orbital shaker (150 rpm) at 50 °C for 48 h. *S. cerevisiae* ethanol red was used for fermentation at 35 °C for 48 h. SSF—enzymes and yeast inoculum were incubated in an orbital shaker (150 rpm) at 35 °C for 72 h. PSSF—enzymatic hydrolysis was conducted at 50 °C during the first 8 h. After which the yeast was added and incubated at 35 °C for 64 h in orbital shaker (150 rpm)	SHF—48.0 SSF—46.2 PSSF—48.3	SHF—0.40 SSF—0.64 PSSF—0.67	Guerrero et al. (2018)

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Banana pseudostem	Acid-catalyzed steam explosion with 2.2% H$_2$SO$_4$ (v/v) at 177 °C and 5 min	SHF—enzymatic hydrolysis at 50 °C during the first 24 h. Then, the bottles were transferred to an orbital shaker (150 rpm) at 50 °C for 48 h. *S. cerevisiae* ethanol red was used for fermentation at 35 °C for 48 h. SSF—enzymes and yeast inoculum were incubated in an orbital shaker (150 rpm) at 35 °C for 72 h. PSSF—enzymatic hydrolysis was conducted at 50 °C during the first 8 h, after which the yeast was added and incubated at 35 °C for 64 h in orbital shaker (150 rpm)	SHF—42.0 SSF—40.1 PSSF—41.8	SHF—0.35 SSF—0.56 PSSF—0.52	

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Sweet sorghum bagasse	Pretreated by 30% H_2O_2 at 25 °C for 24 h and then 10% NaOH at 121 °C for 20 min	SHF—enzymatic hydrolysis was conducted at 50 °C for 72 h at 140. Fermentation with *S. cerevisiae* TISTR 5606 at 30 °C for 9 h. SSF—The enzyme and yeast were incubated at 37 °C, 150 rpm for 72 h	SHF—25.1 SSF—22.3	SHF—0.31 SSF—0.31	Thanapimmetha et al. (2019)
Agave lechuguilla cogollos	Autohydrolysis pretreatment at 1:6 w/v ratio at 190 °C, 200 rpm for 30 min	SHF—enzymatic hydrolysis was conducted at 50 °C and 200 rpm for 24 h. Fermentation at 35 °C and 150 rpm for 12 h. SSF—the SSF were performed for 26 h at 35 °C with both the enzyme and the yeast at the beginning of the process. PSSF—pre-hydrolysis was performed at 50 °C and 200 rpm for 24 h. The temperature was readjusted to 35 °C, inoculated (10% v/v), and incubated at 150 rpm for 12 h	SHF—53.7 SSF—10.4 PSSF—45	SHF—1.49, SSF—0.29, PSSF—1.25,	Morales-Martínez et al. (2017)

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Corn stover	Treated with pure ethylenediamine at 0.8 mL/g corn stover at 130 °C for 20 min. The mixture was poured on a stainless-steel tray and stirred every 5 min at 130 °C for 40 min	The biomass was first pre-hydrolyzed using commercial enzymes, pH 5.4, 250 rpm at 50 °C and for 12 h. After the pre-hydrolysis, temperature was adjusted to 34 °C and yeast added at 150 rpm for 48 h	PSScF—39.8		Qin et al. (2018)
Corn stover	Dry dilute acid pretreatment with 2.5% sulfuric acid at 170 °C for 3 min with agitation. There was no liquid fraction and no detoxification treatment performed after pretreatment	Pre-hydrolysis with enzymes at 50 °C and 200 rpm for 12 h before the inoculation of co-culture yeast. The yeast co-culture contained xylose-utilizing engineered *S. cerevisiae* and inhibitor-tolerant *S. cerevisiae*. The solid loading was 25% (w/w) dry matter loading at pH 5.5 for 72 h during fermentation	PSScF—58.8		Zhu et al. (2016)

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Pinus pinaster stumps	Pretreated with 50 g/L NaOH at 10% (w/v) solids loading at 170 C for 120 min. Then pretreated with 35 wt% ethanol solution and 50 g/L NaOH at 8% (w/v) solids loading at 170 C for 120 min	SSF was performed with a combination of enzymes and *S. cerevisiae (ATCC 26602TM)* at 10 wt% solids and 38 °C for 72 h	SSF—43.9	SSF—1.66	Mendes et al. (2021)
Pinus roxburghi	1% HCl and a biomass loading of 5% (w/v) at 121 °C for 1 h	Enzymatic hydrolysis with cellulases and pectinase at 30 °C and 200 rpm for 72 h. Fermentation with *S. cerevisiae* (MTCC-36) for 24 h at 28 °C	SHF—0.144 g/g biomass		Slathia et al. (2019)
		Enzymatic hydrolysis with cellulases and pectinase at 30 °C and 200 rpm for 72 h. Fermentation with both *S. cerevisiae* (MTCC-36) and *Pichia stipitis* (NCIM-3498) for 24 h at 28 °C	SHF—0.2 g/g biomass		

Table 2 (continued)

Biomass	Pretreatment	Hydrolysis and fermentation	Ethanol yield (g L^{-1})	Ethanol productivity (g L^{-1} h^{-1})	References
Pinus pseudostrobus sawdust	Pretreated with 9.25% HNO$_3$ at 115 °C for 30 min. The samples were washed and treated with 1% NaOH at 90 °C for 30 min	Enzymatic hydrolysis was conducted with an enzyme cocktail at 48 °C and 150 rpm for 72 h. Fermentation was conducted with *S. cerevisiae* at 30 °C and 150 rpm for 40 h	SHF—17.01		Farías-Sánchez et al. (2017)
		A mixture of enzymatic cellulase and *S. cerevisiae* yeast were added to pretreated biomass at 35 °C and 150 rpm for 72 h	SSF—15.0		

3 Current strategies in addressing bioethanol production challenges

3.1 Improvement to individual steps

3.1.1 Pretreatment

Current trends in biomass pretreatment have been focused on using environmentally friendly methods while reducing cost. To ensure that pretreatment is eco-friendly, focus has been placed on that are non-toxic, biodegradable and recyclable. This has promoted the recent interest in deep eutectic solvents (DES) (Ullah et al., 2023). DES are a new class of designer solvents made up of renewable materials found in nature (Amesho et al., 2023; Loong et al., 2021). They are made up of two or more components held together by strong hydrogen bonds. The components are referred to as a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) with respect to their function in the designer solvent. DES are produced by heating a HBA and a HBD together at temperatures below 100 °C for less than 2 h to produce a homogeneous, colourless liquid after cooling to room temperature. They have shown their effectiveness in the removal of lignin from biomass (Ceaser et al., 2023).

There is a variety of natural components that can be used to produce different types of DES. However, acid- and polyalcohol-based DES have shown better effectiveness in biomass delignification (Table 3). Choline chloride (ChCl) is mostly used as a HBA due to its low cost, biodegradability and non-toxicity in combination with a variety of acids or/and polyalcohols as HBDs. Lactic, formic, oxalic and p-coumaric acids are a few among those reported to perform well in biomass pretreatment. Lignin removal from 28 to 99% and almost complete hemicellulose solubilisation have been reported, giving a pretreated cellulose pulp that was saccharified with a 86% of efficiency (Chen et al., 2019a, 2019b, 2019c; Kim et al., 2018; Tan et al., 2018, 2019). In comparison to the other acid-based DES, oxalic acid is toxic and has caustic properties, thereby reducing its applicability (Kohli et al., 2020). Polyalcohols such as ethylene glycol and glycerol have shown lignin removal and hemicellulose solubilisation of up to 88% and 87%, respectively, with a saccharification efficiency of up to 96% (Zhang et al., 2016; Zulkefli et al., 2017). This shows that while the acid-based DES might be more effective in removing lignin and solubilising hemicellulose, the polyalcohol-based DES are better at promoting saccharification of the pretreated biomass.

Recently, there has been a growing interest in the use of ternary DES (TDES). TDES offer flexibility to operate at higher solids loading (up to 30%) without having a detrimental effect on the capacity to extract lignin and hemicellulose from the biomass and the saccharification efficiency (Chen et al., 2019a; Ji et al., 2020; Kandanelli et al., 2018). A comparison of TDES with two acid-based DES as HBDs showed higher performance than DES having only one acid-based HBD (Xing et al., 2018). Yan et al. (2021) later found out that TDES with both a polyalcohol- and acid-based HBD, not only had similar performance as TDES with two acid-based HBDs but also retained more glucan, making them attractive for bioethanol production. Other studies replaced the natural acid-based HBDs with other strong acids such as H_2SO_4, p-toluene sulfonic acid, $AlCl_3$, $FeCl_3$ and $CrCl_3$ (; Chen et al., 2018a, 2018b, 2019a; Ji et al., 2020). However, these replacements either required strong acid supplementation after DES recycling or contained leaching organic acid groups, defeating the purpose of producing a "green" and recyclable pretreatment medium (Chen et al., 2018a, 2018b; Deng et al.,

Table 3 Selected chemical pretreatment methods and their lignin removal and enzymatic hydrolysis efficiency

Chemical pretreatment method	Biomass	Pretreatment conditions	Lignin removal, %	Enzymatic hydrolysis efficiency, %	References
Dilute acid	Wheat straw	3% Sulphuric acid at 140 °C for 60 min	15.6	81.9	Chen et al., (2018a, 2018b)
Alkaline	Sugarcane bagasse	3% NaOH at 50 °C for 4 h	87	72.8	Wang et al. (2019)
	Paddy straw	0.5% NaOH at 100 °C for 30 min	69.9		Gabhane et al. (2020)
	Sugarcane bagasse	2% NaOH at 121 °C for 60 min	79.9	80.5	Fan et al. (2020)
	Wheat straw	2% NaOH at 121 °C for 30 min	84.6	85.4	Chen et al., (2018a, 2018b)
Organic solvent	Wheat straw	65% ethanol at 220 °C for 20 min	46.4	74.6	Chen et al., (2018a, 2018b)
	Wheat straw	65% ethanol at 220 °C for 20 min	46.4	78.3	Chen et al. (2015)
Deep eutectic solvent (DES)	Corncob	ChCl/glycerol at 90 °C for 24 h	71.3	96	Zhang et al. (2016)
	Corncob	ChCl/ethylene glycol at 90 °C for 24 h	87.6	85.3	Zhang et al. (2016)
	Corncob	ChCl/oxalic acid at 90 °C for 24 h	98.5	45.2	Zhang et al. (2016)
	Corncob	ChCl/glycerol at 150 °C for 16 h	71.8	94.9	Procentese et al. (2017)
	Switchgrass	ChCl/p-Coumaric acid at 160 °C for 3 h	60.8	85.7	Kim et al. (2018)
	Herb residues	ChCl/p-Coumaric acid at 160 °C for 5 h	28	84.6	Chen et al., (2019a, 2019b, 2019c)
	Loblolly Pine	ChCl/formic acid at 155 °C for 2 h		70	Lynam et al. (2017)
	Pinus bungeana Zucc	ChCl/lactic acid at 120 °C for 4 h	66.6	45.7	Li et al. (2019)
	Rice straw	ChCl/lactic acid at 60 °C for 12 h	63	36	Kumar et al. (2016)
	Switchgrass	ChCl/ethylene glycol/p-TSA at	59.1	72	Chen et al., (2019a, 2019b, 2019c)
	Miscanthus	ChCl/glycerol at 110 °C for 12 h	81.9	96.2	Hassan and Mutelet (2022)
Microwave-assisted hot water extraction	Aspenwood	Microwave-assisted hot water at 195 °C for 20 min	19.4	77.8	Mihiretu et al. (2017)
Microwave-assisted hot water extraction	Sugarcane trash	Microwave-assisted hot water 194 °C for 15 min	21.4	74.3	Mihiretu et al. (2017)
Microwave-assisted NaOH treatment	Wheat straw	Microwave-assisted 1.5% NaOH for 15 min	69.5	78.5	Tsegaye et al. (2019)

Table 3 (continued)

Chemical pretreatment method	Biomass	Pretreatment conditions	Lignin removal, %	Enzymatic hydrolysis efficiency, %	References
Microwave-assisted organosolv + H2SO4	Mixed sawdust	Microwave-assisted ethanol/water (60:40) and 0.25% H_2SO_4 for 60 min at 175 °C	50	80	Alio et al. (2020)
Microwave-assisted DES	Pinus bungeana Zucc	Microwave-assisted ChCl/lactic acid at 800 W for 10 min	42.8	81.9	Li et al. (2019)
DAP and steam explosion (SE)	Olive stone	DAP at 128 C for 60 min and SE at 195 °C for 5 min		78.1	Padilla-Rascón et al. (2020)
Organosolv steam explosion	Spruce wood chips	52% ethanol and SE at 200 °C for 30 min	76.2	49	Matsakas et al. (2019)
FeCl$_3$ catalysed organosolv pretreatment	Sugarcane bagasse	Treated with 0.05 M FeCl$_3$ and ethanol/water (60:40) at 160 °C for 10 min	59.1	93	Zhang et al. (2018)

2015). It is therefore paramount to revisit and improve the use of natural acid-based and polyalcohol-based HBDs for biomass pretreatment.

DES pretreatments can also be performed in combination or in sequence with other treatments. Li et al. (2019) compared a microwave-assisted DES pretreatment and a conventional heating DES pretreatment using ChCl:lactic acid (1:10) on *Pinus* spp. wood. Microwave-assisted DES pretreatment resulted in less glucan removal (10%) in comparison with conventional heating (19%) due to the reduced reaction time. Despite the high lignin removal (66.6%) by conventional DES pretreatment, the cellulose conversion after enzymatic hydrolysis was only 41.6% while the lower lignin removal (42.8%) by the microwave-assisted pretreatment produced 81.9% cellulose conversion. This was attributed to an improved removal of non- and low-substituted xylan as well as highly-substituted lignin with microwave irradiation, causing morphological changes to the biomass and making cellulose more accessible to enzymatic hydrolysis. Chen and Wan (2018) also achieved lignin removals of 72.2%, 79.60% and 65.2% for switchgrass, corn stover and *Miscanthus*, respectively, using microwave-assisted ChCl: lactic acid pretreatment at 800 W, 152 °C for 45 s. The lignin purity obtained was between 85 and 87% in all cases. Despite the loss of some xylose to the liquid fraction during pretreatment, a twofold, fivefold and twofold increase in sugar yield was observed in comparison with untreated biomass for corn stover, switchgrass and *Miscanthus*, respectively. In addition, the microwave-assisted pretreatment consumed less energy than with conventional heating.

Ji et al. (2020) investigated the use of an oil bath (110 °C, 4 h), microwave (MW) (80 °C, 20 min), ultrasound (US) (room temperature, 30 min) and combinations of ultrasound (room temperature, 30 min) and oil bath (110 °C,4 h) and ultrasound (room temperature, 30 min) and microwave (80 °C, 20 min) on garlic skin (GS) and green onion root (GOR) for several binary DES (BDES) and TDES. Lignin removal in the binary DES improved in the order ChCl:oxalic acid < ChCl:urea < ChCl:glycerol for both GS and GOR. A similar trend was observed when $AlCl_3·6H_2O$ was added to the various BDES to form TDES. Similar cellulose contents were obtained irrespective of the type of BDES or TDES used for GS while for GOR the ChCl:glycerol (:$AlCl_3·6H_2O$) gave the highest cellulose content in the pretreated biomass. The effects on TDES were significantly higher than in BDES. An evaluation of MW pretreatment gave high lignin removal while combined US-MW further improved the removal of lignin and the cellulose content of the pretreated biomass. For instance, MW processing with ChCl:glycerol:$AlCl_3·6H_2O$ gave a lignin removal and a cellulose content in the GOR residue of 87.6% and 40.9%, respectively, while US-MW gave 92.3% and 50.3%. Furthermore, the nearly fourfold increase in the yield of total reducing sugars with US-MW over MW alone was attributed to the significant reduction in the crystallinity of the residue (8.1 vs. 16.9) by the action of the ultrasonication.

Biomass pretreatment is targeted at improving the hydrolysis step and therefore more consideration should be given to the influence of extent of delignification on hydrolysis. Procentese et al. (2015) reported that using ChCl:glycerol (1:2), 30% delignification, 83% glucose and 50% xylose hydrolysis can be achieved giving a 70% TSY. Biomass delignification can be improved with an increase in the acid content of the DES (Kumar et al., 2016). However, a 100% delignification is not essential to obtain optimum hydrolysis efficiency. It was determined that about 65–70% of lignin in biomass could be removed easily as far as lignin redeposition was avoided (Bhagia et al., 2018). This was supported by the findings by Zhang et al. (2016), who reported that even if lignin removal increased from 64.7 to 93.1% in corncob when the acid content in DES was augmented (ChCl: lactic acid from 1:2 to 1:15), the enzymatic hydrolysis yield barely improved (79.1–83.5%). Excessive delignification could also cause the destruction of cellulose structure creating a

less favourable fibre morphology for enzymatic hydrolysis, thereby leading to a reduction in hydrolysis yields (Lin et al., 2020).

Thus far, DES pretreated biomass have been reported to contain very little inhibitors that could affect hydrolysis or fermentation. Procentese et al. (2017) observed that ChCl:glycerol pretreatment between 80 and 150 °C for 3 to 16 h produced acetic acid (0.12–0.19 g/L) and furfural (0.11–0.15 g/L) as the main inhibitors of the fermentation microorganisms, but these concentrations were too low to affect the fermentation process. The same researchers also reported low acetic acid (0.09–0.16 g/L) and furfural (0.12 g/L) concentrations for ChCl:urea whereas acetic acid (0.01–0.08 g/L), furfural (0.01–0.04 g/L) and HMF (0.02–0.09 g/L) were the inhibitors formed with ChCl:imidazole (Procentese et al., 2015). Pretreating rice straw with ChCl:lactic acid at 120 °C for 3 h produced 0.28 g/L furfural (Huang et al., 2020). Given the threshold of *S. cerevisiae* for inhibitors such as acetic acid (0.5 g/L), HMF (0.1 g/L) and furfural (0.5 g/L), the values reported above can be considered to be well below those required to avoid significant inhibition (Baral & Shah, 2014). Phenolic based inhibitors such as p-coumaric, ferulic, benzoic, vanillic and syringic acids were produced when lignin degraded during pretreatments at elevated temperature and extended time (Xu et al., 2018). Nevertheless, the concentrations were still low (< 1 mM) at these severe conditions. Therefore, DES-based pretreatments imply low inhibitor production and no detoxification requirements after pretreatment.

3.1.2 Mechanocatalysis and hydrolysis

To reduce the cost associated with the enzymes, researchers have suggested either enzyme recycling, or the substitution of the enzymatic hydrolysis with a process based on mechanocatalysis combined with autohydrolysis (Arthur et al., 2021; Calcio Gaudino et al., 2021; Chen et al., 2021a, 2021b). Enzyme recycling processes such as ultrafiltration are generally costly and have enzyme losses close to 20%, and the stability of the recycled enzymes may be affected by the recovery and recycling operations (Chen et al., 2021a, 2021b). This has made mechanocatalysis an alternative to be considered. Mechanocatalysis involves the combination of mechanical treatment with a catalyst to improve solubility and reactivity. Mechanical treatment, especially ball milling can reduce the crystallinity of cellulose, improving the rate of hydrolysis (Sitotaw et al., 2021; Wu et al., 2021). Mechanical treatment was reported to improve the hydrolysis rate by 23–59% while the product yield was increased by 5–25% (Aditiya et al., 2016). A classic mechanocatalysis process is made up of three steps, (1) dosage and impregnation of small amounts of a strong acid (pKa ≤ − 3) in diethyl ether, (2) removal of the diethyl ether by vacuum evaporation, and (3) ball milling at room temperature to convert water-insoluble polymers to water-soluble oligomeric products (WSP).

The addition of solid acids or mineral acids even before the mechanical treatment can depolymerise lignocellulosic biomass to obtain 10–20% WSP (Käldström et al., 2014b). Mechanocatalysis of cellulose causes a conformational change to the pyranic ring structure facilitating protonation and fracturing of the β-1,4-glucosidic bonds to produce WSP. The crystalline regions of cellulose are totally converted into amorphous regions resulting in the disappearance of the crystalline peak at 22.7° in an x-ray diffractogram even at low acid loadings (0.25 mmol/g) and milling time (20 min) (Yu et al., 2016). Although it has been suggested that there is no acid loss during the mechanocatalysis process, other researchers have shown that about 11% of the acid can be lost after the removal of diethyl ether (Dong et al., 2017; Yu et al., 2016). This was attributed to water/solvent vapour being a carrier for

part of the acid during vacuum evaporation and the cleavage of diethyl ether by the acid to alcohol, an esterification reaction and vacuum evaporation of the product (Dong et al., 2017).

Yu et al. (2016) observed that without acid-impregnation, only 2% WSP were produced from cellulose after 60 min of ball milling. Acid-impregnation with 0.25 mmol/g H_2SO_4 gave 64% and 90% WSP after 20 and 60 min, respectively. Further, increasing the acid concentration to 0.60 mmol/g resulted in 82% and 99% WSP after 20 and 60 min, respectively while a further increase to 1.0 mmol/g resulted in a limited increase in WSP. Given that the major contention with mechanical treatments is the energy consumption, a convenient combination of acid load and milling time has to be established for each biomass type to minimize pretreatment cost.

The produced WSP can then be further hydrolysed in water at 100–150 °C for 0.5–2 h to give the desired monomeric sugars and lignin, easily separable by filtration (Chen et al., 2021a, 2021b; Käldström et al., 2014a). Cellobiose, a dimer of glucose can be almost totally converted into glucose at low acid concentrations (Yu et al., 2016). The mechanical forces applied were suggested to help reduce the cellobiose to glucose energy barrier by almost five-fold for hydrolysis (Amirjalayer et al., 2019).

Dong et al. (2017) conducted a study comparing direct acid impregnation before ball milling (DAMC) with the conventional mechanocatalysis (CMC) processes for pinewood sawdust. In the DAMC process, about 95% water-soluble products were obtained within 45 min milling time at high acid concentration (0.45 mol/kg biomass) or within 75 min at low acid concentration (0.20 mol/kg biomass). However, the highest total reducing sugars (TRS) yield (30%) after water hydrolysis at 100 °C was obtained at 0.45 mol/kg for 30 min during DAMC while for the CMC process a similar yield was obtained with 0.30 mol/kg for 30 min. The CMC process helped to soften the biomass, allowing easy cleavage into oligomers and monomers at lower acid concentration and reduced time.

Raw cellulose, acid-impregnated and unimpregnated mechanocatalysed cellulose were hydrolysed at 150 °C for 30 min to determine the effectiveness of the treatments on glucose recovery (Yu et al., 2016). Glucose yield was 6% for raw cellulose, while ball milling for 40 min led to 28% glucose recovery. Cellulose impregnated before ball milling with 0.25 mmol/g and 0.50 mmol/g of H_2SO_4 for 40 min gave a similar glucose recovery (87%). Dilute acid hydrolysis conducted on wood biomass at 150 °C with 0.25 wt% H_2SO_4 for 30 min, 96% galactose, 99% arabinose, 72% xylose, 74% mannose and 7% glucose were obtained representing a 32% TSY. Ball milling for 40 min combined with dilute acid hydrolysis at the same conditions gave 99% galactose, 99% arabinose, 99% xylose, 92% mannose and 16% glucose, for a total 45% TSY. Additional impregnation of the wood biomass with 0.25 mmol/g of acid before ball milling for 40 min, followed by aqueous hydrolysis at 150 °C gave 95% galactose, 99% arabinose, 98% xylose, 90% mannose and 92% glucose, representing 94% TSY. From this study, ball milling combined with dilute acid hydrolysis was found to be an effective method to obtain high hemicellulose-based monomers, whereas mechanocatalysis allowed an almost total recovery of the C5 and C6 saccharides forming cellulose and hemicellulose in biomass.

After obtaining 100% WSP from beechwood mechanocatalysed with 0.9 mmol/g H_2SO_4, performing hydrolysis at 140 °C for 1 h gave 84% glucose, 89% xylose and 3.9% cellobiose (Käldström et al., 2014b). The hydrolysis of WSP obtained from beechwood, pinewood and sugarcane bagasse produced 88–92% glucose, 93–98% xylose, and 3.6–8.0% cellobiose yields after water hydrolysis at 140 °C for 1 h (Käldström et al., 2014a). The effects of temperature on the hydrolysis of WSP into glucose, xylose and cellobiose was then determined. Xylose was the first monomeric sugar to be formed at temperatures

20–30 °C lower than glucose. The highest xylose yields were obtained at 120, 135 and 140 °C for sugarcane bagasse (99%), beechwood (94%) and pinewood (99%), respectively whereas the highest glucose yields were at 140 and 145 °C for sugarcane bagasse (95%), beechwood (92%) and pinewood (88%). Although the hydrolysis followed the same trend as in DAP, the lower hydrolysis temperature (120–140 °C) in comparison with two-step DAP (180 and 210 °C) ensured that lesser inhibitors were produced.

Hydrolysis of α-cellulose, sugarcane bagasse, beechwood and pinewood at 140 °C produced furfural as the main inhibitor (Käldström et al., 2014a, 2014b; Meine et al., 2012). Käldström et al. (2014a) found that since the highest xylose yield was obtained at lower temperature than glucose, part of the xylose formed was converted to furfural as the temperature was increased to increase the production of glucose. At 120 and 135 °C for sugarcane bagasse and beechwood, respectively the furfural yield did not exceed 1.5% of the initial xylan present; however, at 140 °C the furfural yield was 5.0 and 5.7%, respectively. The authors suggested an initial separation of the xylose fraction at the optimal temperatures before proceeding to the hydrolysis to produce glucose as a solution. However, the solubility of both products in water makes this difficult to accomplish.

Unlike in most treatment processes, where the lignin presence affects the process efficiency, the rate of depolymerisation is not affected by the biomass lignin content during the mechanocatalysis process (Käldström et al., 2014b). The main disadvantage of mechanocatalysis is the energy expenditure, which is similar to that of a standard mechanical treatment. However, it has been reported that for the same equipment, increasing the biomass load from grams to kilograms results in a reduction in energy expenditure per mass of biomass (Kaufman Rechulski et al., 2015). Also, the low acid requirement (0.25–1.0 mmol/g cellulose) to achieve more than 90% conversion of cellulose to glucose sugar implies less neutralisation and detoxification (Kessler et al., 2020; Shrotri et al., 2013). This makes it an attractive process for large scale applications (Shrotri et al., 2017).

3.1.3 Fermentation

To address the inefficient hydrolysis issue due to low temperature in SSF, presaccharification and simultaneous saccharification and fermentation (PSSF) was developed (Table 4). In this process, saccharification is conducted first at 50 °C, and then temperature is reduced below 37 °C to perform the SSF. Stoichiometric yield (76.1%) and ethanol productivity of 0.39 g/L/h were obtained after 72 h for PSSF while lower results were observed with SSF (56.3%, 0.31 g/L/h, 72 h) and SHF (63.4%, 0.31 g/L/h, 82 h) (Thanapimmetha et al., 2019). Guerrero et al. (2018) also observed higher PSSF theoretical yield (86.6%) and ethanol productivity (0.67 g/L/h) after 72 h in comparison with SHF (85.9%, 0.40 g/L/h after 120 h) and SSF (82.8%, 0.64 g/L/h after 72 h). Interestingly, Morales-Martínez et al. (2017) obtained 97.5% theoretical yield for SHF, 96.1% for PSSF and 77.9% for SSF. The authors have shown that the effectiveness of the PSSF is based on the effectiveness of the presaccharification stage giving higher yields and productivity than the SSF process.

The presence of pentose sugars such as xylose in the hydrolysate obtained after saccharification presents an additional opportunity to increase the yield of ethanol. Processes involving a mixed culture of hexose- and pentose-fermenting microbes are known as simultaneous saccharification and co-fermentation (SSCF) (Table 4). Another advantage of SSCF is the absence of sugar feedback inhibition (Robak & Balcerek, 2018). The

Table 4 Advantages and disadvantages of various hydrolysis and saccharification configurations

Configuration	Advantages	Disadvantages
Separate hydrolysis and fermentation (SHF)	Possible to conduct hydrolysis and fermentation under optimal treatment conditions to obtain optimum ethanol yields	Requires separate vessels for hydrolysis and fermentation Requires cooling of hydrolysate after hydrolysis before undergoing fermentation Glucose accumulation during hydrolysis can inhibit activity of cellulase enzymes Pentose sugars are not converted to ethanol
Simultaneous saccharification and fermentation (SSF)	Saccharification and fermentation steps are conducted in the same vessel at the same time Reduction of total ethanol production time No cellulase inhibition	Saccharification is not conducted at optimal conditions Pentose sugars are not converted to ethanol
Prehydrolysis and simultaneous saccharification and fermentation (PSSF)	Hydrolysis is conducted at optimal temperature before SSF Reduction of total ethanol production time in comparison with SHF No cellulase inhibition	Requires cooling of hydrolysate after hydrolysis before undergoing fermentation Pentose sugars are not converted to ethanol
Separate hydrolysis and co-fermentation (SHCF)	Possible to conduct hydrolysis and fermentation under optimal treatment conditions to obtain optimum ethanol yields Fermentation of both hexose and pentose sugars	Requires cooling of hydrolysate after hydrolysis before undergoing fermentation Requires separate vessels for hydrolysis and fermentation
Simultaneous saccharification and co-fermentation (with prehydrolysis) (SSCF)	No cellulase inhibition Fermentation of both hexose and pentose sugars	Requires cooling of hydrolysate after prehydrolysis before undergoing SSF

advancements in genetic engineering have spearheaded this process. Qin et al. (2018) used an engineered xylose-fermenting strain of *S. cerevisiae* to produce ethanol from corn stover pretreated with ethylenediamine. Presaccharification was first performed for 12 h at 50 °C on the pretreated biomass before SSCF at 34 °C for 96 h. Increasing the solids loading had a higher detrimental effect on glucan than on xylan conversion. Ethanol yields of 75.4%, 81.2% and 80.9% were obtained at biomass loads of 6%, 7.5% and 9%. Wirawan et al. (2020) recently studied the continuous co-fermentation of alkali-pretreated sugarcane bagasse using *Zymomonas mobilis* immobilized on polyvinyl alcohol (PVA) beads and suspended *Pichia stipites*. An ethanol yield of 81.8% and a productivity of 0.705 g/L/h were obtained with SSCF, while those were 70.65% and 1.868 g/L/h with SHCF. It was observed that even if ethanol yields could be kept high for a short time, cell activity decayed for *Pichia stipites* and therefore the addition of nutrient solution was needed. Ko et al. (2018) reported that engineered yeast strains were less tolerant to the inhibitors produced during the production of lignocellulosic ethanol. Inhibitors such as acetic acid and phenolics had a stronger effect on the strains of engineered yeast during SSCF because of their higher concentration than in SHCF. Zhu et al. (2016) developed a co-culture of a xylose-fermenting and inhibitor-tolerant *S. cerevisiae* strain for bioethanol production from DAP corn stover. In comparison with either the solo inhibitor-tolerant or xylose-fermenting strains, the co-culture SSCF improved ethanol yields by 21.2% and 40.0%, respectively. Furthermore, presaccharification for 3 h before SSCF resulted in 89.0% ethanol yield.

As already explained (Sect. 2.3), the low operating temperature used in SSF and SSCF does not ensure hydrolysis and optimal sugar yield. To address this challenge, thermophilic microorganisms that can be used at temperatures 50–70 °C have become a focus of interest for these processes (Di Donato et al., 2019). Usage of thermophilic microbes makes the cooling step from the ethanol production process unnecessary. Native microbes of the genera *Clostridium* and *Thermoanaerobacter* have been found successful in addressing this issue. Orlygsson (2012) used *Clostridium strain* AK1 to ferment cellulose hydrolysate from various biomasses at 45 °C to obtain a 66.75% yield of ethanol. Fermenting avicel with *C. thermocellum* at 60 °C gave yields of 3.0 g/L which was later increased by co-culturing with *C. thermolacticum* to 4.19 g/L representing 75% of the stoichiometric yield (Scully & Orlygsson, 2015). *Thermoanaerobacter* sp. DBT-IOCX2 was able to ferment both glucose and xylose at 70 °C giving yields of 83.57% and 91.12% (Singh et al., 2018). The anaerobic bacterium species *Thermoanaerobacter italicus* Pentocrobe 411 fermented complex sugars of glucose and xylose from non-detoxified DAP wheat straw, birch, sugarcane bagasse, oil palm empty fruit brunch and frond, cardboard and mixed biowaste at 66 °C (Andersen et al., 2015). Ethanol yields obtained were 92–100% of the theoretical ethanol yields for all biomasses. Introduction of thermophilic microbes has therefore led to modest gains in ethanol yield while reducing the formation of side products.

3.2 Cost reduction

3.2.1 Pretreatment

The cost of biomass pretreatment is of a major factor in the economic feasibility of lignocellulosic ethanol (Wang & Lee, 2021). Pretreatment alone accounts for up to one-third of the total production cost in a bioethanol conversion plant and it is therefore necessary to find ways to reduce the cost (Rodrigues Gurgel da Silva et al., 2018). A study of various traditional pretreatment techniques concluded that DAP was the most favourable,

boasting both the highest economic and environmental margins (Rodrigues Gurgel da Silva et al., 2018). Environmental margins of liquid hot water, ammonia fibre explosion and organosolv pretreatment were 30–40% lower than DAP while SE was the lowest by 47%. In terms of the economic margins liquid hot water and ammonia fibre explosion are below 50% of DAP, while SE and organosolv do not exceed 25% of the DAP's economic margin.

Research has focused on the reuse of the pretreatment chemicals as means to reduce cost. Although some of the studies conducted using mineral acids and alkalis such as H_2SO_4 and NaOH have recycled and reused the waste liquor, the limited number of cycles in which the chemicals can be reused and the low efficiency of the liquor during consecutive pretreatment cycles are still challenges to be resolved (Chen et al., 2021a, 2021b). Similar recycling and loss of pretreatment efficiency issues at extended recycle runs have been reported for DES. Nevertheless, recycling and reuse of the pretreatment chemicals for the first two to three recycle runs present comparable results (Chen et al., 2018b, 2019b). Furthermore, higher cost reductions can be obtained with DES given that it is cheaper than NaOH and that 95% of the DES can be recycled in comparison to only 70% of the NaOH (Cha et al., 2016; Kim et al., 2018; Li et al., 2018; New et al., 2019). This implies DES is the most cost-effective pretreatment chemical when recycled and reused.

Few studies have been conducted on the cost of DES-based biomass pretreatments. Peng et al. (2021) recently determined the cost of ChCl:urea and ChCl:lactic acid pretreatments on the production of bioethanol from rice straw. The DES pretreatment, the solids load and temperature had little impact on the total capital investment required. However, the total operating cost was lower with ChCl:lactic acid than with ChCl:urea. Further analysis of both pretreatments showed that the highest contributors to the operating cost were steam, cooling water and electricity. This was due to the cost of the recovery and purification of the DES by distillation to remove other solvents such as water, ethanol, acetone and impurities. The authors also concluded that a higher solids loading would be beneficial in reducing the cost of the DES recycling.

3.2.2 Hydrolysis

As stated in Sect. 3.1.2, enzymatic hydrolysis is the most expensive step in the bioethanol production process due to the cost of enzymes (Larnaudie et al., 2019; Mesa et al., 2016). The cost of the enzymes was estimated to account for up to 71% of the total hydrolysis cost and 35% of the lignocellulose bioethanol cost (Baral & Shah, 2017; Wang et al., 2022). Although, treatments such as dilute acid hydrolysis are relatively cheaper in comparison with enzymatic treatment, one of their major drawbacks is the production of inhibitors.

The use of mechanocatalysis/water hydrolysis presents a potential solution to addressing the cost issues associated with enzymatic hydrolysis. The low acid concentration, cheaper chemicals, higher solid loading and reduced reaction time could serve well in this situation. A comparison of a mechanocatalysis/water hydrolysis production of ethanol with a National Renewable Energy Laboratory's (NREL) cellulosic ethanol process involving DAP and enzymatic hydrolysis, showed that the mechanocatalysed process led to a 30% lower ethanol cost and a 5% reduction in greenhouse gases than the NREL ethanol process (Nguyen, 2017). The mechanocatalysis/water hydrolysis step can either be used on untreated biomass and/or pretreated biomass. For a pretreated biomass, the savings are mainly centred on the enzyme cost and time for the hydrolysis step. Although pretreating the biomass with a "green" method such as DES would increase both the biomass crystallinity and cost, it would reduce the presence of lignin, improve the porosity of

biomass, and potentially reduce the milling time and acid required, thus avoiding the need for detoxification. Furthermore, the hemicelluloses extracted during the pretreatment could be recovered and recycled into mechanocatalysis step, reducing sugar losses.

Conventional mechanocatalysis/water hydrolysis can be conducted directly on the raw biomass. The advantages of this pretreatment are the potential savings on the cost of product separation and recycling, as well as the savings on enzyme and time in the hydrolysis step. However, the detoxification required after hydrolysis and its high water consumption become factors to be considered (Baral & Shah, 2014, 2017; Kessler et al., 2020). Llano et al. (2021) analysed various detoxification processes based on technical, economic, environmental and social factors. The analysis showed that from a techno-economic point of view overliming was the best detoxification method. However, when all the factors were considered for an ethanol biorefinery, activated carbon detoxification was the most suitable. This agrees with other studies reported that activated carbon and calcium hydroxide were the most effective detoxification methods in an ethanol biorefinery (Kordala et al., 2021).

3.2.3 Fermentation

The yield of bioethanol during fermentation depends on the ability of the yeast to convert the available monosaccharides. The introduction of pentose fermenting microbes has ensured maximum use of the monomeric sugars available in the hydrolysate, improving process economics. The cost of equipment and installations for handling and treating the pentose containing hydrolysate increases the capital cost. Vasconcelos et al. (2020) noticed that despite the higher energy consumption and increased equipment quantity, co-fermenting the pentose LCB sugar hydrolysates from acid treatments with the hexose sugars, lowered the ethanol production cost thereby improving the economic performance.

The cost of yeast and its preparation for the fermentation process also needs to be considered. Recycling the yeast can lower this cost. The difference in density between yeast cells and LCB makes centrifugation an adequate method. At low speeds, the denser LCB can be obtained while at high speeds the lighter yeast cells can be recovered (Matano et al., 2013). Yeast recycling rises the inoculation rate, reduces the amount of sugar consumed by yeast grow and improves the ability of the microbes to adapt and resist inhibitors, thereby increasing the yield of bioethanol (Chen et al., 2021a, 2021b). Matano et al. (2013) observed a decreasing cell recycle (88.3%, 56.4% and 5.8%) with an increase in centrifugal force (20 g, 50 g and 100 g, respectively) in a two-phase yeast cell recycling after fermentation. At a centrifugal force of 50 g with five consecutive recycling cycles after fermentation, a wild strain of *S. cerevisiae* gave an average of 71.9% of ethanol while an engineered strain gave 86.3%. Cell recycling was found to be an effective means of reducing the cost of inoculum preparation. A high ethanol yield of 90.3% was obtained after six fermentation-recycle runs when *S. cerevisiae* was engineered to tolerate multiple stress and elevated temperature was used (Hama et al., 2018). Therefore, although recycling is beneficial with both wild and engineered yeast strains, the use of engineered strains improves ethanol yield.

3.3 Current advances in process configurations

The advances in the individual process steps have led to improved process configurations, with less waste treatment requirements and overall cost. Traditionally, process
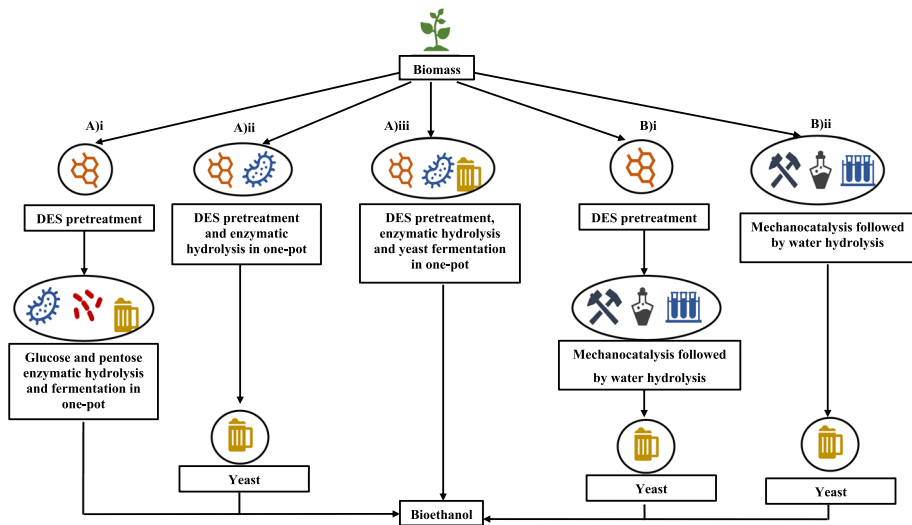
Fig. 3 **a i**, **ii** and **iii** Current improvements in bioethanol production process, **b i** and **ii** envisioned modifications in bioethanol production process

improvements such as SSF or SSCF are applied in place of SHF. The current advancements of these options deal with the inclusion of a pretreatment step to minimise the cost of washing. Such a configuration would result in the "one-pot" (OP) system, thereby reducing the number of required vessels for the entire bioethanol production process (Fig. 3).

Advances on the use of DES in biomass processing have created opportunities by combining the pretreatment with the hydrolysis and fermentation steps (Fig. 3a iii). However, the main issue with this has been the stability and activity of the hydrolytic enzymes in DES, as well as the effect of DES on the grow and activity of the fermentation yeasts (Wahlström et al., 2016; Xu et al., 2018). For instance, Wahlström et al. (2016) reported that although the various DES tested performed well in delignifying the biomass and improving the enzymatic hydrolysis of the pretreated and washed material, the DES affected both the enzyme stability and performance, thus reducing the yield of sugars in a OP system.

Gunny et al. (2015) investigated the effects of ChCl:glycerol and ChCl:ethylene glycol on the activity of cellulase. About 90% of the original cellulase activity was maintained in the presence of 10–20% v/v DES in citrate buffer at 30 °C. This was attributed to the epoxidase hydrolases activity present in polyalcohol-based DES such as glycerol and ethylene glycol. Also, the DES-cellulase system enhanced glucose production (> 183%) and reduced energy consumption (127.7 J/g) in comparison with an alkaline system (133% and 159.7 J/g, respectively). Similar glucose production rates were observed as concentration of solids was increased from 5 to 20%, both in ChCl:ethylene glycol and ChCl:glycerol. However, at 30% solids, the higher viscosity of glycerol in comparison to ethylene glycol resulted in ca. half the glucose production rate at similar conditions due to reduced ionic mobility influencing both the reaction thermodynamics and the kinetics.

A substitution of the *Aspergillus* sp. cellulase with an halophilic *Aspergillus* sp. cellulase in the same DES-cellulase system showed improved cellulase activity (Nagoor Gunny et al., 2019). Addition of 15% v/v ChCl:glycerol to 1% cellulase improved the enzyme

activity by 30% and 40% within 1 h. At 24 and 48 h, there was no difference between the cellulase activity with and without DES whereas exceeding 20% v/v DES reduced cellulase activity. Tests conducted with microcrystalline cellulose and rice husk showed a two to three-fold increase in glucose yields at 20% v/v DES as compared to the system without DES.

A recent study conducted by Huang et al. (2020) investigated a OP system for simultaneous pretreatment and enzymatic hydrolysis (Fig. 3a ii). ChCl:lactic acid was used in pretreating rice straw (15% solids loading) at 120 °C for 3 h, after which the slurry was diluted with citrate buffer and the pH adjusted to 4.8 before the addition of enzymes. An increase in DES concentration had a negative effect on the cellulase activity and sugar yield. Comparison of the traditional solid–liquid-separation (SLS) process for consecutive pretreatment and hydrolysis with the simultaneous pretreatment and hydrolysis OP approach showed that the latter produced ca. 1.6-folds higher TSY than the SLS process at the same conditions. The OP glucose yield was 69.0%, comparable to the 66.6% obtained by SLS, however the OP xylose yield was 70.0% while for SLS was only 22.4%. The difference could be ascribed to the xylan loss during pretreatment affecting the SLS xylose yield. The OP process was over 8 times more energy efficient than the SLS process at the same conditions due to the avoidance of the SLS between pretreatment and hydrolysis. Furthermore, at the capital cost for the OP system was 5.5 times lower than for the SLS system. However, the profit obtained for the SLS system ($0.20/kg) was similar to the profit from the OP system ($0.19/kg). The main reason for the slightly higher profit was due to the lower purity of the lignin recovered in the OP process, which was only 33.3%, well below the purity of lignin from the SLS system (83.7%).

Xu et al. (2018) studied the effects of various DES on the grow of *S. cerevisiae* (BY4741). The best results were obtained with ChCl:glycerol with a yeast growth above the control test, while all the other DES systems had lower yeast growths (Fig. 3a iii). The yeast growth increased in the order ChCl:acid-based < ChCl:basic-based < ChCl:polyalcohol-based DES. When the acid-based DES (ChCl:oxalic acid and ChCl:levulinic acid) were used, there was almost no growth of yeast. Corn stover was later used as a feedstock in an OP system to produce bioethanol through consecutive hydrolysis and fermentation at different temperature. In the one-pot system, ChCl:glycerol pretreatment was first performed at 180 °C for 2 h to remove over 60% of the lignin. Pre-saccharification of the pretreated slurry was conducted at 50 °C for 24 h by adding citrate buffer to obtain 5 wt% DES, followed by an SSF operation at 37 °C for 48 h with *S. cerevisiae*. This configuration produced 84.5% glucose yield that gave a 77.5% ethanol conversion yield. A further study done to ascertain if it was possible to co-ferment xylose and glucose using an engineered *S. cerevisiae* strain (JBEI-9009), showed that the strain could tolerate up to 10 wt% ChCl:glycerol without significantly impairing yeast growth. A longer lag phase was observed in the presence of DES as compared to the control. However, both systems with and without DES, reached similar xylose consumption levels.

4 Techno-economic analysis (TEA)

The choice between single or integrated technologies for lignocellulosic biomass impacts energy consumption, economic feasibility, and environmental safety, considering technical and economic factors. A comprehensive TEA and life cycle assessment (LCA) is crucial to determine the sustainability of DES in biorefining, especially for emerging treatments

at low technology readiness levels (TRL). DES-based biorefineries must showcase technical scalability, economic viability, and environmental attractiveness to be considered sustainable compared to alternative processes. Economic indicators, including capital and operational expenditures, net present value, and payback period, are assessed based on simulation mass and energy balance results. Evaluation of technical viability and economic profitability of emerging technologies, like DES, involves TEA with simulation scaling-up exercises. However, TEA studies on DES-based biorefineries, critical for commercial scalability, are currently limited.

Recent studies shed light on the economic feasibility of DES-based biorefinery plants. Despite the critical impact of DES recycling time on operating costs, all studies found economic advantages in using DES. Kumar et al. (2020) presented the inaugural TEA for a natural 1 ton/day DES-based biorefinery, emphasizing benefits like recyclability and non-toxicity. In the study, key economic parameters such as payback period, net present value (NPV) and internal rate of return (IRR) were considered. The three scenarios considered in the study were (1) a traditional biorefinery where agricultural residues are converted into bioethanol by enzymatic saccharification and fermentation (2) a biorefinery with silica or biogas production (3) a biorefinery with value-added products such as cellulose, lignin, xylan and silica. Scenario 3 was observed to be the best scenario for the biorefinery with a positive NPV of $1.4 million, > 100% IRR, and a payback of < 2 years, highlighting the sustainability and economic advantages of multi-product biorefineries.

Additionally, Peng et al. (2021) and Zang et al. (2020) conducted TEAs for bioethanol production and co-production of various chemicals, respectively. Peng et al. (2021) investigated the use of ChCl:lactic acid and ChCl:urea as DES for pretreatment of rice straw to produce bioethanol and lignin as a by-product. The acidic-based DES resulted in a higher bioethanol yield compared to the urea-based DES giving a minimum selling price (MSP) of 2128.1 $/ton compared to 3049.9 $/ton. The MSP of ethanol obtained was lower than 3250 $/ton obtained by pretreating sugarcane bagasse with phosphoric acid or 2225 $/ton obtained by pretreating corn stover with liquid hot water (da Silva et al., 2016; van Rijn et al., 2018). In comparison to dilute sulfuric acid pretreatment on corn stover (660.4 $/ton) and wheat straw (1545.4 $/ton), the MSP was high (Hossain et al., 2019; Zhao et al., 2015). The authors concluded that the higher MSP with DES was due to lower lignin removal efficiency and a less than 80% enzymatic saccharification rate. Nevertheless, the use of high solid loading, 20% in DES pretreatment led to reduction in capital and operating costs.

The lignin removal efficiency could be increased to about 90% with the use of ChCl:formic acid DES obtaining lignin with a high purity (Ceaser et al., 2023). This could help reduce the MSP by improving the co-product, lignin extraction and purity. To address the low enzymatic saccharification rate, Huang et al. (2020) found that conducting the pretreatment and saccharification in one-pot improved the sugar yield to 75.7% when compared to the 49.9% by a traditional solid–liquid separation process. However, there was a need to double the enzyme dosage which would result in a further increase in operating cost.

Although promoting high solid loading has been suggested to enhance the minimum selling price (MSP) of bioethanol from DES pretreatment, attention should also be directed to the enzymatic saccharification process. For instance, Peng et al. (2021) observed a 23.4% reduction in MSP with a 20% increase in enzymatic hydrolysis efficiency after DES pretreatment. The presence of chemicals in the pretreated biomass often inhibits enzymatic saccharification, and replacing this step with mechanochemical hydrolysis could improve cellulose saccharification. Yu et al. (2016) achieved a 94% monomeric sugar recovery from woody biomass through mechanocatalytic treatment coupled with hydrolysis. However,

there is a lack of published literature on the techno-economic analysis (TEA) and life cycle assessment (LCA) of mechanocatalyzed and hydrolyzed lignocellulosic biomass. The focus in this field has primarily been on lignin-first extraction and oligosaccharide production rather than monosaccharides for bioethanol. Nevertheless, studies by Jin et al. (2020) and Liu et al. (2020) suggest that mechano-catalytic biomass exhibits similar ethanol titers to glucose, with the latter achieving a 75% glucose yield and an economically feasible ethanol titer of 42 g/L at a high solid loading of 92 mg/mL. These findings indicate the potential of this treatment technique as a cost-effective replacement for enzymatic saccharification.

5 Implications for current and future bioethanol production

Current advances in the production of bioethanol from LCB aim at increasing the yields of ethanol using green methods. Energy consumption, time and costs are reduced. The introduction of DES has become a turning point for the pretreatment process. High solids loading are applicable with TDES without losing delignification efficiency. These green and recyclable chemicals have provided an opportunity to overcome many of the issues related to more conventional pretreatments. Furthermore, the ability to recover the components of lignocellulose from the DES solutions reduces biomass loses. Finally, the low inhibitor production excludes the need for detoxification, further reducing pretreatment cost and saving energy and time.

The ability to combine DES with hydrolytic enzymes and fermentation microbes without significantly affecting their activities is of significance to the feasibility of the bioethanol production process. This has been the main consideration in the OP configurations. The reduction in energy and cost due to the exclusion of the SLS steps between the individual processes could improve process economics. However, more studies are required to fully understand the OP system and ascertain the feasibility on the recovery of other products, and the recycling and reuse of the DES and the fermentative microorganisms.

Mechanocatalysis is an effective tool to reduce cost from both biomass pretreatment and hydrolysis. The high solids loading used in mechanocatalysis is advantageous for bioethanol production. Figure 3b i and ii shows two probable bioethanol production routes based on the current advances for individual treatment steps. Given that a breakthrough in pretreatment has been achieved with the introduction of DES, the following process to tackle to reduce costs significantly is enzymatic hydrolysis. A probable future approach involves replacement of the enzymatic hydrolysis with mechanocatalysis/water hydrolysis. It is envisioned that this process could reduce treatment time from 24–72 h to less than 6 h, while reducing cost and achieving equivalent sugar yields. Potential bottlenecks for the implementation of this process are the formation of fermentation inhibitors, and the success of its implementation as a one-pot operation.

Figure 3b i involves a DES pretreatment, mechanocatalysis, water hydrolysis and fermentation for bioethanol production whereas Fig. 3b ii involves mechanocatalysis, water hydrolysis and fermentation. Each path has its own merits and demerits. Although Fig. 3b i involves more steps, the DES pretreatment would reduce the lignin content of LCB, potentially reducing the time and energy required for the mechanocatalysis process. Additionally, given that the hemicellulose extracted during DES is recoverable, it could be recycled back into the process to improve ethanol yields. In Fig. 3b ii, the exclusion of DES pretreatment implies less energy consumption and more cost savings, however the

potential of inhibitor formation increases making a detoxification step necessary to ensure that fermentation is not negatively affected.

Based on the current status of bioethanol production and the new foreseeable alternatives, there is clearly a need for additional research to achieve optimized processes that reduce the current cost of bioethanol production significantly. Also, comprehensive techno-economic evaluation of the different processes should guide the design of compact and effective plant designs. Co-production of other products such as xylitol, biogas, furfural, HMF, hydrogen, electricity and phenolic compounds should be considered as well to improve the overall economics and sustainability (Preethi et al., 2021; Queiroz et al., 2022; Zang et al., 2020).

6 Bibliometry

The literature study showed a number of publications in English related to the key subjects within this review from 1st January 2010 till the 18th January 2024. The data was compiled from Web of Science whiles the relevant associated terms were composed using the VOSViewer application. The data obtained was exported 1000 entries at a time, based on the total number. A bibliometric analysis mapping was generated for the data for the network visualization. The analysis was conducted based on co-occurrence, keywords and a fractional counting technique which allowed for the visualization of all significant terms linked to the key subjects.

A combination of the keywords "lignocellulose or lignocellulosic biomass" and "bioethanol" on the Web of Science database showed a total of 6005 results while an addition of the term "techno-economic" reduced the total results to 197 (Fig. 4a, b). In Fig. 4c, shows that the research into the techno-economics of lignocellulosic biomass production of bioethanol has eventually become widespread into various research areas lately. The research therefore stands the chance of being quickly scaled up to make it industrially relevant.

A combination of lignocellulose biomass and deep eutectic solvent showed a total of 888 results with 87% of the publications in the last 5 years (Fig. 5a, b). Addition of key words such as pretreatment, bioethanol and techno-economic consecutively gave results of 665, 79 and 4 publications. Among the 4 publications on the techno-economics of lignocellulosic biomass pretreated with deep eutectic solvent to produce bioethanol 2 were review articles while the other 2 were research articles. This shows that this aspect of research is still in its preliminary stages and would require more studies to reach an industrial ready level.
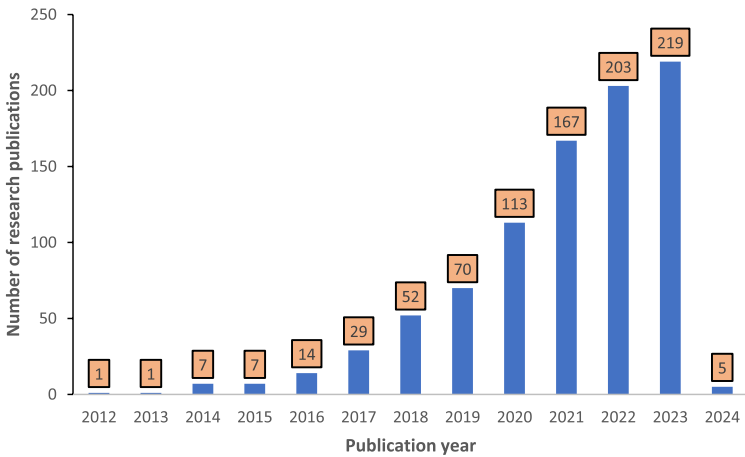
(A)

(B)
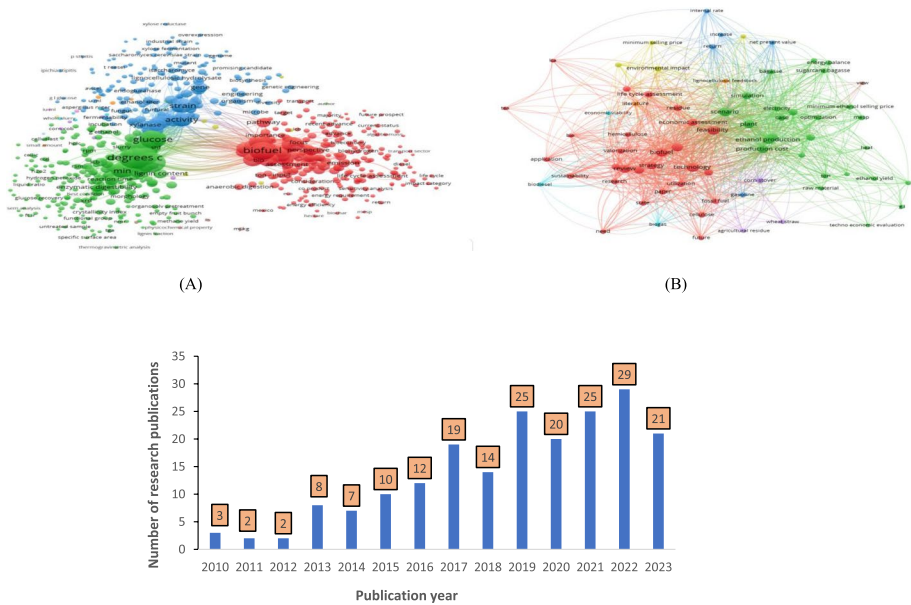
Fig. 4 Network visualization of terms associated with **a** lignocellulosic biomass and bioethanol, **b** lignocellulosic biomass, bioethanol and techno-economics. **c** Number of publications and publication year associated with the term lignocellulosic biomass, bioethanol and techno-economics

Combining keywords like lignocellulose biomass and mechanocatalysis yielded 65 results published between 2013 and 2023 (Fig. 6a, b). Further addition of keywords "hydrolysis or saccharification," "bioethanol," and "pretreatment" resulted in 46, 10, and 8 data entries, respectively. Despite its linkage to various research fields, there has been no techno-economic assessment in this subject area. Conducting more experiments is crucial to determine the industrial feasibility of this process for large-scale applications. Interestingly, a combination of keywords related to emerging technologies, including lignocellulose biomass, deep eutectic solvent pretreatment, and mechanocatalysis, did not yield any results. However, these emerging techniques could complement each other, potentially enhancing the economic attractiveness of bioethanol production.
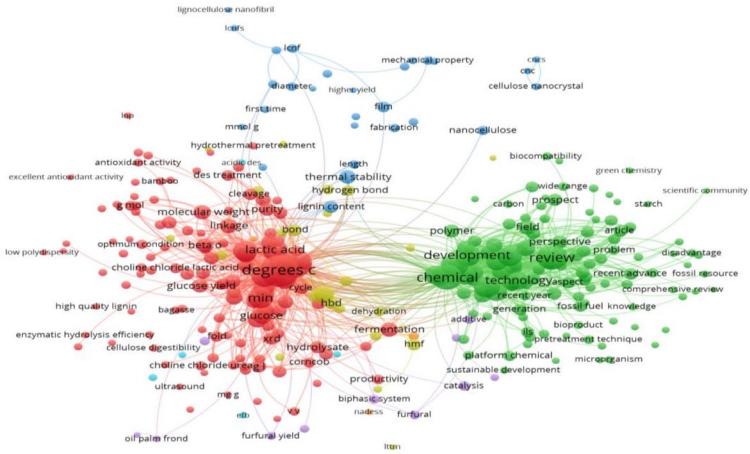
(A)

(B)

Fig. 5 Network visualization of terms associated with **a** lignocellulosic biomass deep eutectic solvents, **b** number of publications and publication year associated with the term lignocellulosic biomass and deep eutectic solvents
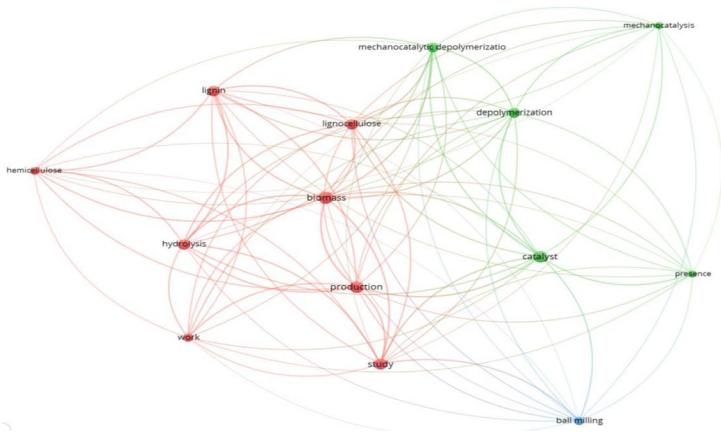
(A)
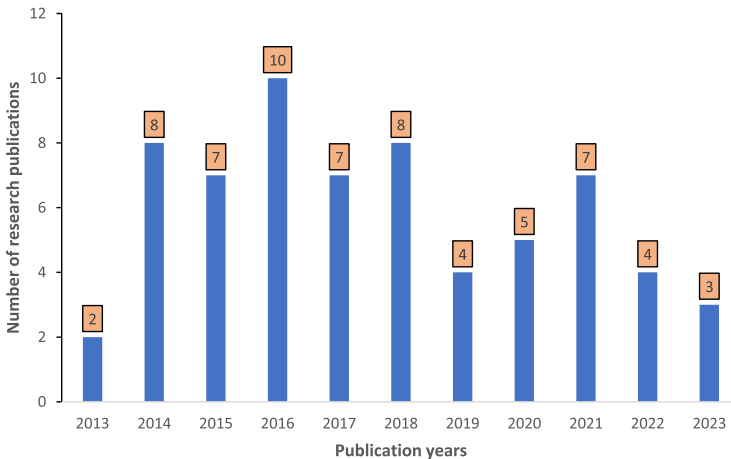
(B)

Fig. 6 Network visualization of terms associated with **a** lignocellulosic biomass and mechanocatalysis, **b** number of publications and publication year associated with the term lignocellulosic biomass and mechanocatalysis

7 Conclusions

The shift from 1 to 2G bioethanol feedstocks is anticipated, but lignocellulosic biomass (LCB) faces competitiveness challenges compared to conventional 1G biomass. This concise review explores challenges and ongoing solutions for 2G bioethanol production, focusing on pretreatment, hydrolysis, and fermentation. Addressed challenges include reagent toxicity, waste generation, recyclability of chemicals and enzymes, cost, and process configuration. Deep eutectic solvents (DES) are highlighted as environmentally

friendly and cost-effective for pretreatment, offering advantages like up to 30% solid loading, less than 10% cellulose loss, extraction of high purity lignin, and chemical recyclability. Mechanocatalysis/water hydrolysis is proposed as an alternative to enzymatic hydrolysis, operating at 100% solid loading with negligible inhibitors and similar sugar yields. The review emphasizes the implementation of thermophilic ethanologenic microbes for enhanced fermentation yields and suggests integrating these strategies into an effective process configuration to make 2G bioethanol production competitive on an industrial scale.

Data availability statement This review is entirely based on published information and does not contain any unpublished data. All the sources are included in the references of the manuscript.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

References

Abo, B. O., Gao, M., Wang, Y., Wu, C., Ma, H., & Wang, Q. (2019). Lignocellulosic biomass for bioethanol: An overview on pretreatment, hydrolysis and fermentation processes. *Reviews on Environmental Health, 34*, 57–68. https://doi.org/10.1515/reveh-2018-0054

Aditiya, H. B., Mahlia, T. M. I., Chong, W. T., Nur, H., & Sebayang, A. H. (2016). Second generation bioethanol production: A critical review. *Renewable and Sustainable Energy Reviews, 66*, 631–653. https://doi.org/10.1016/j.rser.2016.07.015

Althuri, A., & Banerjee, R. (2019). Separate and simultaneous saccharification and fermentation of a pretreated mixture of lignocellulosic biomass for ethanol production. *Biofuels, 10*, 61–72. https://doi.org/ 10.1080/17597269.2017.1409059

Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology, 101*, 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093

Amesho, K. T. T., Cheng, P. C., Chang, K. L., Peng, Y. P., Jhang, S. R., & Lin, Y. C. (2022). Microwave-assisted deep eutectic solvents/dimethyl sulfoxide system for efficient valorization of sugar bagasse waste into platform chemicals: A biorefinery approach for circular bioeconomy. *Bioresource Technology, 363*, 127969. https://doi.org/10.1016/J.BIORTECH.2022.127969

Amesho, K. T. T., Lin, Y. C., Mohan, S. V., Halder, S., Ponnusamy, V. K., & Jhang, S. R. (2023). Deep eutectic solvents in the transformation of biomass into biofuels and fine chemicals: A review. *Environmental Chemistry Letters*. https://doi.org/10.1007/s10311-022-01521-x

Amirjalayer, S., Fuchs, H., & Marx, D. (2019). Understanding the mechanocatalytic conversion of biomass: A low-energy one-step reaction mechanism by applying mechanical force. *Angewandte Chemie, 131*, 5286–5289. https://doi.org/10.1002/ange.201811091

Andersen, R. L., Jensen, K. M., & Mikkelsen, M. J. (2015). Continuous ethanol fermentation of pretreated lignocellulosic biomasses, waste biomasses, molasses and syrup using the anaerobic, thermophilic bacterium Thermoanaerobacter italicus pentocrobe 411. *PLoS ONE, 10*, 1–16. https://doi.org/10.1371/journal.pone.0136060

Arthur, W., Diedericks, D., Coetzee, G., Van Rensburg, E., & Görgens, J. F. (2021). Kinetic modelling of cellulase recycling in paper sludge to ethanol fermentation. *Journal of Environmental Chemical Engineering*. https://doi.org/10.1016/j.jece.2021.105981

Baral, N. R., & Shah, A. (2014). Microbial inhibitors: Formation and effects on acetone-butanol-ethanol fermentation of lignocellulosic biomass. *Applied Microbiology and Biotechnology, 98*, 9151–9172. https://doi.org/10.1007/s00253-014-6106-8

Baral, N. R., & Shah, A. (2017). Comparative techno-economic analysis of steam explosion, dilute sulfuric acid, ammonia fiber explosion and biological pretreatments of corn stover. *Bioresource Technology, 232*, 331–343.

Beig, B., Riaz, M., Raza Naqvi, S., Hassan, M., Zheng, Z., Karimi, K., Pugazhendhi, A., Atabani, A. E., & Thuy Lan Chi, N. (2020). Current challenges and innovative developments in pretreatment of lignocellulosic residues for biofuel production: A review. *Fuel, 287*, 119670. https://doi.org/10.1016/j.fuel.2020.119670

Bhagia, S., Pu, Y., Evans, B. R., Davison, B. H., & Ragauskas, A. J. (2018). Hemicellulose characterization of deuterated switchgrass. *Bioresource Technology, 269*, 567–570. https://doi.org/10.1016/j.biortech.2018.08.034

Bibi, F., Ilyas, N., Saeed, M., Shabir, S., Shati, A. A., Alfaifi, M. Y., Amesho, K. T. T., Chowdhury, S., & Sayyed, R. Z. (2023). Innovative production of value-added products using agro-industrial wastes via solid-state fermentation. *Environmental Science and Pollution Research, 30*(60), 125197–125213. https://doi.org/10.1007/S11356-023-28765-6

Binod, P., Janu, K. U., Sindhu, R., & Pandey, A. (2011). *Hydrolysis of lignocellulosic biomass for bioethanol production* (1st ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-385099-7.00010-3

Broda, M., Yelle, D. J., & Serwá Nska, K. (2022). Bioethanol production from lignocellulosic biomass—Challenges and solutions. *Molecules, 27*, 8717. https://doi.org/10.3390/MOLECULES27248717

Calcio Gaudino, E., Cravotto, G., Manzoli, M., & Tabasso, S. (2021). Sono- and mechanochemical technologies in the catalytic conversion of biomass. *Chemical Society Reviews, 50*, 1785–1812. https://doi.org/10.1039/d0cs01152e

Calderon, O. R., & Arantes, V. (2019). A review on commercial—Scale high—Value products that can be produced alongside cellulosic ethanol. *Biotechnology for Biofuels, 12*, 1–58. https://doi.org/10.1186/s13068-019-1529-1

Carvalho, A., & Ishikawa, L. (2019). The economic viability of second-generation ethanol in coming years (pp. 101–108). https://doi.org/10.1007/978-3-319-93488-4_12.

Ceaser, R., & Chimphango, A. F. A. (2021). Comparative analysis of physical and functional properties of cellulose nanofibers isolated from alkaline pre-treated wheat straw in optimized hydrochloric acid and enzymatic processes. *International Journal of Biological Macromolecules, 171*, 331–342. https://doi.org/10.1016/j.ijbiomac.2021.01.018

Ceaser, R., Rosa, S., Montané, D., Constantí, M., & Medina, F. (2023). Optimization of softwood pretreatment by microwave-assisted deep eutectic solvents at high solids loading. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2022.128470

Cha, Y. L., Yang, J., Seo, S. I., An, G. H., Moon, Y. H., You, G. D., Lee, J. E., Ahn, J. W., & Lee, K. B. (2016). Alkaline twin-screw extrusion pretreatment of Miscanthus with recycled black liquor at the pilot scale. *Fuel, 164*, 322–328. https://doi.org/10.1016/j.fuel.2015.10.006

Chen, H., Zhao, J., Hu, T., Zhao, X., & Liu, D. (2015). A comparison of several organosolv pretreatments for improving the enzymatic hydrolysis of wheat straw: substrate digestibility, fermentability and structural features. *Applied Energy*, *150*, 224–232. https://doi.org/10.1016/j.apenergy.2015.04.030

Chen, H., & Fu, X. (2016). Industrial technologies for bioethanol production from lignocellulosic biomass. *Renewable and Sustainable Energy Reviews, 57*, 468–478. https://doi.org/10.1016/J.RSER.2015.12.069

Chen, J., Zhang, B., Luo, L., Zhang, F., Yi, Y., Shan, Y., Liu, B., Zhou, Y., Wang, X., & Lü, X. (2021a). A review on recycling techniques for bioethanol production from lignocellulosic biomass. *Renewable and Sustainable Energy Reviews, 149*, 111370. https://doi.org/10.1016/j.rser.2021.111370

Chen, L., Yu, Q., Wang, Q., Wang, W., Qi, W., Zhuang, X., Wang, Z., & Yuan, Z. (2019a). A novel deep eutectic solvent from lignin-derived acids for improving the enzymatic digestibility of herbal residues from cellulose. *Cellulose, 8*, 1–13. https://doi.org/10.1007/s10570-018-2190-8

Chen, P., Shrotri, A., & Fukuoka, A. (2021b). Synthesis of cello-oligosaccharides by depolymerization of cellulose: A review. *Applied Catalysis, a: General, 621*, 118177. https://doi.org/10.1016/j.apcata.2021.118177

Chen, Z., Bai, X., Lusi, A., & Wan, C. (2018a). High-solid lignocellulose processing enabled by natural deep eutectic solvent for lignin extraction and industrially relevant production of renewable chemicals. *ACS Sustainable Chemistry & Engineering, 6*, 12205–12216. https://doi.org/10.1021/acssuschemeng.8b02541

Chen, Z., Jacoby, W. A., & Wan, C. (2019b). Ternary deep eutectic solvents for effective biomass deconstruction at high solids and low enzyme loadings. *Bioresource Technology, 279*, 281–286. https://doi.org/10.1016/j.biortech.2019.01.126

Chen, Z., Reznicek, W. D., & Wan, C. (2018b). Deep eutectic solvent pretreatment enabling full utilization of switchgrass. *Bioresource Technology, 263*, 40–48. https://doi.org/10.1016/j.biortech.2018.04.058

Chen, Z., Sun, Y., & Wan, C. (2019c). Effects of alkaline hydrogen peroxide treatment on cellulose accessibility of switchgrass pretreated by acidic deep eutectic solvent. *Cellulose, 26*, 9439–9446. https://doi.org/10.1007/s10570-019-02759-5

Chen, Z., & Wan, C. (2018). Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment. *Bioresource Technology, 250*, 532–537. https://doi.org/10.1016/j.biortech.2017.11.066

da Silva, A. R. G., Torres Ortega, C. E., & Rong, B. G. (2016). Techno-economic analysis of different pretreatment processes for lignocellulosic-based bioethanol production. *Bioresource Technology, 218*, 561–570. https://doi.org/10.1016/J.BIORTECH.2016.07.007

Delmas, G. H., Banoub, J. H., & Delmas, M. (2022). Lignocellulosic biomass refining: A review promoting a method to produce sustainable hydrogen, fuels, and products. *Waste and Biomass Valorization, 13*, 2477–2491. https://doi.org/10.1007/s12649-021-01624-6

Deng, W., Zhang, Q., & Wang, Y. (2015). Catalytic transformations of cellulose and its derived carbohydrates into 5-hydroxymethylfurfural, levulinic acid, and lactic acid. *Science China: Chemistry, 58*, 29–46. https://doi.org/10.1007/s11426-014-5283-8

Di Donato, P., Finore, I., Poli, A., Nicolaus, B., & Lama, L. (2019). The production of second generation bioethanol: The biotechnology potential of thermophilic bacteria. *Journal of Cleaner Production, 233*, 1410–1417. https://doi.org/10.1016/j.jclepro.2019.06.152

Dong, Y., Haverinen, J., Tuuttila, T., Jaakkola, M., Holm, J., Leveque, J. M., & Lassi, U. (2017). Rapid one-step solvent-free acid-catalyzed mechanical depolymerization of pine sawdust to high-yield water-soluble sugars. *Biomass and Bioenergy, 102*, 23–30. https://doi.org/10.1016/j.biombioe.2017.03.026

Eggeman, T., & Elander, R. T. (2005). Process and economic analysis of pretreatment technologies. *Bioresource Technology, 96*, 2019–2025. https://doi.org/10.1016/j.biortech.2005.01.017

El-Zawawy, W. K., Ibrahim, M. M., Abdel-Fattah, Y. R., Soliman, N. A., & Mahmoud, M. M. (2011). Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production. *Carbohydrate Polymers, 84*, 865–871. https://doi.org/10.1016/j.carbpol.2010.12.022

European Commission. (2024). *Renewable energy directive* [WWW Document]. Retrieved 9 January, 2024, from https://energy.ec.europa.eu/topics/renewable-energy/renewable-energy-directive-targets-and-rules/renewable-energy-directive_en.

Fan, Z., Lin, J., Wu, J., Zhang, L., Lyu, X., Xiao, W., Gong, Y., Xu, Y., & Liu, Z. (2020). Vacuum-assisted black liquor-recycling enhances the sugar yield of sugarcane bagasse and decreases water and alkali consumption. *Bioresource Technology, 309*, 123349. https://doi.org/10.1016/j.biortech.2020.123349

Farías-Sánchez, J. C., Velázquez-Valadez, U., Pineda-Pimentel, M. G., López-Miranda, J., Castro-Montoya, A. J., Carrillo-Parra, A., & Rutiaga-Quiñones, J. G. (2017). Simultaneous saccharification and fermentation of pine sawdust (Pinus pseudostrobus L.) pretreated with nitric acid and sodium hydroxide

for bioethanol production. *BioResources, 12*(1), 1052–1063. https://doi.org/10.15376/biores.12.1.1052-1063

Fernandes, M. C., Ferro, M. D., Paulino, A. F., Mendes, J. A., Gravitis, J., Evtuguin, D. V., & Xavier, A. M. (2015). Enzymatic saccharification and bioethanol production from Cynara cardunculus pretreated by steam explosion. *Bioresource Technology, 186*, 309–315. https://doi.org/10.1016/j.biortech.2015.03.037

Fernandes, M. C., Ferro, M. D., Paulino, A. F. C., Chaves, H. T., Evtuguin, D. V., & Xavier, A. M. R. B. (2018). Comparative study on hydrolysis and bioethanol production from cardoon and rockrose pretreated by dilute acid hydrolysis. *Industrial Crops and Products, 111*, 633–641. https://doi.org/10.1016/j.indcrop.2017.11.037

Flach, B., Lieberz, S., & Bolla, S. (2019). GAIN report—EU biofuels annual 2019, Global Agricultural Information Network.

Gabhane, J., Prince William, S. P. M., Vaidya, A. N., Mahapatra, K., & Chakrabarti, T. (2011). Influence of heating source on the efficacy of lignocellulosic pretreatment—A cellulosic ethanol perspective. *Biomass and Bioenergy, 35*, 96–102. https://doi.org/10.1016/j.biombioe.2010.08.026

Gabhane, J., Kumar, S., & Sarma, A. K. (2020). Effect of glycerol thermal and hydrothermal pretreatments on lignin degradation and enzymatic hydrolysis in paddy straw. *Renewable Energy, 154*, 1304–1313. https://doi.org/10.1016/j.renene.2020.03.035

Galbe, M., & Zacchi, G. (2007). Pretreatment of lignocellulosic materials for efficient bioethanol production. *Advances in Biochemical Engineering/biotechnology*. https://doi.org/10.1007/10_2007_070

Gandam, P. K., Chinta, M. L., Pabbathi, N. P. P., Baadhe, R. R., Sharma, M., Thakur, V. K., Sharma, G. D., Ranjitha, J., & Gupta, V. K. (2022). Second-generation bioethanol production from corncob—A comprehensive review on pretreatment and bioconversion strategies, including techno-economic and lifecycle perspective. *Industrial Crops and Products, 186*, 115245. https://doi.org/10.1016/j.indcrop.2022.115245

Guerrero, A. B., Ballesteros, I., & Ballesteros, M. (2018). The potential of agricultural banana waste for bioethanol production. *Fuel, 213*, 176–185. https://doi.org/10.1016/j.fuel.2017.10.105

Gunny, A. A. N., Arbain, D., Nashef, E. M., & Jamal, P. (2015). Applicability evaluation of deep eutectic solvents-cellulase system for lignocellulose hydrolysis. *Bioresource Technology, 181*, 297–302. https://doi.org/10.1016/j.biortech.2015.01.057

Gurgel, R., da Silva, A., Errico, M., & Rong, B. G. (2018). Techno-economic analysis of organosolv pretreatment process from lignocellulosic biomass. *Clean Technologies and Environmental Policy, 20*, 1401–1412. https://doi.org/10.1007/s10098-017-1389-y

Hama, S., Kihara, M., Noda, H., & Kondo, A. (2018). Development of cell recycle technology incorporating nutrient supplementation for lignocellulosic ethanol fermentation using industrial yeast Saccharomyces cerevisiae. *Biochemical Engineering Journal, 137*, 23–29. https://doi.org/10.1016/j.bej.2018.05.007

Hamelinck, C. N., Van Hooijdonk, G., & Faaij, A. P. C. (2005). Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle- and long-term. *Biomass and Bioenergy, 28*, 384–410. https://doi.org/10.1016/j.biombioe.2004.09.002

Hassan, E. S. R., & Mutelet, F. (2022). Evaluation of miscanthus pretreatment effect by Choline chloride based Deep Eutectic solvents on bioethanol production. *Bioresource Technology, 345*, 126460. https://doi.org/10.1016/j.biortech.2021.126460

Hossain, M. S., Theodoropoulos, C., & Yousuf, A. (2019). Techno-economic evaluation of heat integrated second generation bioethanol and furfural coproduction. *Biochemical Engineering Journal, 144*, 89–103. https://doi.org/10.1016/J.BEJ.2019.01.017

Huang, Z. J., Feng, G. J., Lin, K. P., Pu, F. L., Tan, Y. M., Tu, W. C., Han, Y. L., Hou, X. D., Zhang, H. M., & Zhang, Y. (2020). Significant boost in xylose yield and enhanced economic value with one-pot process using deep eutectic solvent for the pretreatment and saccharification of rice straw. *Industrial Crops and Products, 152*, 112515. https://doi.org/10.1016/j.indcrop.2020.112515

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., & Dudgeon, D. (2011). Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: Dilute-acid pretreatment and enzymatic hydrolysis of corn stover. *National Renewable Energy Laboratory (NREL)*. https://doi.org/10.2172/1013269

Ji, Q., Yu, X., Yagoub, A. E. G. A., Chen, L., & Zhou, C. (2020). Efficient removal of lignin from vegetable wastes by ultrasonic and microwave-assisted treatment with ternary deep eutectic solvent. *Industrial Crops and Products, 149*, 112357. https://doi.org/10.1016/j.indcrop.2020.112357

Jin, T., Käldström, M., Benavides, A., Rechulski, M. D. K., & Jarboe, L. R. (2020). Utilization of mechanocatalytic oligosaccharides by ethanologenic Escherichia coli as a model microbial cell factory. *AMB Express*. https://doi.org/10.1186/s13568-020-0965-4

Käldström, M., Meine, N., Farès, C., Rinaldi, R., & Schüth, F. (2014a). Fractionation of 'water-soluble lignocellulose' into C5/C6 sugars and sulfur-free lignins. *Green Chemistry, 16*, 2454–2462. https://doi.org/10.1039/x0xx00000x

Käldström, M., Meine, N., Farès, C., Schüth, F., & Rinaldi, R. (2014b). Deciphering "water-soluble lignocellulose" obtained by mechanocatalysis: New insights into the chemical processes leading to deep depolymerization. *Green Chemistry, 16*, 3528–3538. https://doi.org/10.1039/c4gc00004h

Kandanelli, R., Thulluri, C., Mangala, R., Rao, P. V. C., Gandham, S., & Velankar, H. R. (2018). A novel ternary combination of deep eutectic solvent-alcohol (DES-OL) system for synergistic and efficient delignification of biomass. *Bioresource Technology, 265*, 573–576. https://doi.org/10.1016/j.biortech.2018.06.002

Kaufman Rechulski, M. D., Käldström, M., Richter, U., Schüth, F., & Rinaldi, R. (2015). Mechanocatalytic depolymerization of lignocellulose performed on hectogram and kilogram scales. *Industrial and Engineering Chemistry Research, 54*, 4581–4592. https://doi.org/10.1021/acs.iecr.5b00224

Kessler, M., Ahorsu, R., Medina, F., Constantı, M., & Rinaldi, R. (2020). An integrated cellulase-free approach to produce sugars from lignocellulose at high quality for fermentation processes. *Chemie Ingenieur Technik*. https://doi.org/10.1002/cite.202055127

Kim, K. H., Dutta, T., Sun, J., Simmons, B., & Singh, S. (2018). Biomass pretreatment using deep eutectic solvent from lignin derived phenols. *Green Chemistry, 20*, 809–815. https://doi.org/10.1039/C7GC03029K

Ko, J. K., Jung, J. H., Altpeter, F., Kannan, B., Kim, H. E., Kim, K. H., Alper, H. S., Um, Y., & Lee, S. M. (2018). Largely enhanced bioethanol production through the combined use of lignin-modified sugarcane and xylose fermenting yeast strain. *Bioresource Technology, 256*, 312–320. https://doi.org/10.1016/j.biortech.2018.01.123

Kohli, K., Katuwal, S., Biswas, A., & Sharma, B. K. (2020). Effective delignification of lignocellulosic biomass by microwave assisted deep eutectic solvents. *Bioresource Technology, 303*, 122897. https://doi.org/10.1016/j.biortech.2020.122897

Kordala, N., Lewandowska, M., & Bednarski, W. (2021). Effect of the method for the elimination of inhibitors present in Miscanthus giganteus hydrolysates on ethanol production effectiveness. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-020-01255-2

Kumar, A. K., Parikh, B. S., & Pravakar, M. (2016). Natural deep eutectic solvent mediated pretreatment of rice straw: Bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. *Environmental Science and Pollution Research, 23*, 9265–9275. https://doi.org/10.1007/s11356-015-4780-4

Kumar, A. K., Sharma, S., Dixit, G., Shah, E., Patel, A., & Boczkaj, G. (2020). Techno-economic evaluation of a natural deep eutectic solvent-based biorefinery: Exploring different design scenarios. *Biofuels, Bioproducts and Biorefining, 14*, 746–763. https://doi.org/10.1002/bbb.2110

Kumari, D., & Singh, R. (2018). Pretreatment of lignocellulosic wastes for biofuel production: A critical review. *Renewable and Sustainable Energy Reviews, 90*, 877–891. https://doi.org/10.1016/j.rser.2018.03.111

Larnaudie, V., Ferrari, M. D., & Lareo, C. (2019). Techno-economic analysis of a liquid hot water pretreated switchgrass biorefinery: Effect of solids loading and enzyme dosage on enzymatic hydrolysis. *Biomass and Bioenergy, 130*, 105394. https://doi.org/10.1016/j.biombioe.2019.105394

Li, A., Hou, X., Lin, K., Zhang, X., Fu, M., & Ioeng, J. B. I. B. (2018). Rice straw pretreatment using deep eutectic solvents with different constituents molar ratios: Biomass fractionation, polysaccharides enzymatic digestion and solvent reuse. *Journal of Bioscience and Bioengineering, 126*, 1–9. https://doi.org/10.1016/j.jbiosc.2018.03.011

Li, P., Zhang, Q., Zhang, X., Zhang, X., Pan, X., & Xu, F. (2019). Subcellular dissolution of xylan and lignin for enhancing enzymatic hydrolysis of microwave assisted deep eutectic solvent pretreated Pinus bungeana Zucc. *Bioresource Technology, 288*, 121475. https://doi.org/10.1016/j.biortech.2019.121475

Li, X., Shi, Y., Kong, W., Wei, J., Song, W., & Wang, S. (2022). Improving enzymatic hydrolysis of lignocellulosic biomass by bio-coordinated physicochemical pretreatment—A review. *Energy Reports, 8*, 696–711. https://doi.org/10.1016/j.egyr.2021.12.015

Lin, W., Xing, S., Jin, Y., Lu, X., Huang, C., & Yong, Q. (2020). Insight into understanding the performance of deep eutectic solvent pretreatment on improving enzymatic digestibility of bamboo residues. *Bioresource Technology, 306*, 123163. https://doi.org/10.1016/j.biortech.2020.123163

Liu, X., Liu, H., Yan, P., Mao, L., Xu, Z., & Zhang, Z. C. (2020). Mechanocatalytic synergy for expedited cellulosic ethanol production compatible with integrated biorefinery. *ACS Sustainable Chemistry & Engineering, 8*, 2399–2408. https://doi.org/10.1021/acssuschemeng.9b06018

Llano, T., Rueda, C., Dosal, E., Andrés, A., & Coz, A. (2021). Multi-criteria analysis of detoxification alternatives: Techno-economic and socio-environmental assessment. *Biomass and Bioenergy, 154*, 106274. https://doi.org/10.1016/j.biombioe.2021.106274

Loong, C., Liang, K., Zi, A., Ku, E., Lai, B., Chin, F., Sow, S., Lock, M., Wai, K., Chun, A., Loy, M., & Herng, Y. (2021). Recent advances in green solvents for lignocellulosic biomass pretreatment: Potential of choline chloride (ChCl) based solvents. *Bioresource Technology, 333*, 125195. https://doi.org/10.1016/j.biortech.2021.125195

Lynam, J. G., Kumar, N., & Wong, M. J. (2017). Deep eutectic solvents' ability to solubilize lignin, cellulose, and hemicellulose; thermal stability; and density. *Bioresource Technology, 238*, 684–689.https://doi.org/10.1016/j.biortech.2017.04.079

Maroušek, J. (2013). Pretreatment of sunflower stalks for biogas production. *Clean Technologies and Environmental Policy, 15*, 735–740. https://doi.org/10.1007/S10098-012-0548-4/METRICS

Maroušek, J., Itoh, S., Higa, O., Kondo, Y., Ueno, M., Suwa, R., Komiya, Y., Tominaga, J., & Kawamitsu, Y. (2012). The use of underwater high-voltage discharges to improve the efficiency of Jatropha curcas L. biodiesel production. *Biotechnology and Applied Biochemistry, 59*, 451–456. https://doi.org/10.1002/BAB.1045

Matano, Y., Hasunuma, T., & Kondo, A. (2013). Cell recycle batch fermentation of high-solid lignocellulose using a recombinant cellulase-displaying yeast strain for high yield ethanol production in consolidated bioprocessing. *Bioresource Technology, 135*, 403–409. https://doi.org/10.1016/j.biortech.2012.07.025

Matsakas, L., Raghavendran, V., Yakimenko, O., Persson, G., Olsson, E., Rova, U., & Christakopoulos, P. (2019). Lignin-first biomass fractionation using a hybrid organosolv–Steam explosion pretreatment technology improves the saccharification and fermentability of spruce biomass. *Bioresource Technology, 273*, 521–528. https://doi.org/10.1016/j.biortech.2018.11.055

Meine, N., Rinaldi, R., & Schüth, F. (2012). Solvent-free catalytic depolymerization of cellulose to water-soluble oligosaccharides. *Chemsuschem, 5*, 1449–1454. https://doi.org/10.1002/cssc.201100770

Mendes, V. T. C., Moreira, R., Portugal, A., Graça V. S. Carvalho, M., (2021). Biorefining of pinus pinaster stumpwood for ethanol production and lignin recovery. *Chemical Engineering & Technology, 44*, 1043–1050.https://doi.org/10.1002/ceat.202000447

Mesa, L., López, N., Cara, C., Castro, E., González, E., & Mussatto, S. I. (2016). Techno-economic evaluation of strategies based on two steps organosolv pretreatment and enzymatic hydrolysis of sugarcane bagasse for ethanol production. *Renewable Energy, 86*, 270–279. https://doi.org/10.1016/j.renene.2015.07.105

Mihiretu, G. T., Brodin, M., Chimphango, A. F., Øyaas, K., Hoff, B. H., & Görgens, J. F. (2017). Single-step microwave-assisted hot water extraction of hemicelluloses from selected lignocellulosic materials—A biorefinery approach. *Bioresource Technology, 241*, 669–680. https://doi.org/10.1016/j.biortech.2017.05.159

Mikulski, D., Kłosowski, G., Menka, A., & Koim-Puchowska, B. (2019). Microwave-assisted pretreatment of maize distillery stillage with the use of dilute sulfuric acid in the production of cellulosic ethanol. *Bioresource Technology, 278*, 318–328. https://doi.org/10.1016/j.biortech.2019.01.068

Morales-Martínez, T. K., Díaz-Blanco, D. I., Rodríguez-de la Garza, J. A., Morlett-Chávez, J., Castro-Montoya, A. J., Quintero, J., Aroca, G., & Rios-González, L. J. (2017). Assessment of different saccharification and fermentation configurations for ethanol production from Agave lechuguilla. *BioResources, 12*, 8093–8105. https://doi.org/10.15376/biores.12.4.8093-8105

Nagoor Gunny, A. A., Arbain, D., Javed, M., Baghaei-Yazdi, N., Gopinath, S. C. B., & Jamal, P. (2019). Deep eutectic solvents-halophilic cellulase system: An efficient route for in situ saccharification of lignocellulose. *Process Biochemistry, 81*, 99–103. https://doi.org/10.1016/j.procbio.2019.03.003

New, E. K., Wu, T. Y., Tien Loong Lee, C. B., Poon, Z. Y., Loow, Y. L., Wei Foo, L. Y., Procentese, A., Siow, L. F., Teoh, W. H., Nik Daud, N. N., Jahim, J. M., & Mohammad, A. W. (2019). Potential use of pure and diluted choline chloride-based deep eutectic solvent in delignification of oil palm fronds. *Process Safety and Environment Protection, 123*, 190–198. https://doi.org/10.1016/j.psep.2018.11.015

Nguyen, F. (2017). *A techno-economic analysis of solid acid mechanocatalysis for biomass solubilization in the production of cellulosic ethanol*. Princeton University.

Ocreto, J. B., Chen, W.-H., Ubando, A. T., Park, Y.-K., Sharma, A. K., Ashokkumar, V., Ok, Y. S., Kwon, E. E., Rollon, A. P., & De Luna, M. D. G. (2021). A critical review on second- and third-generation

bioethanol production using microwaved-assisted heating (MAH) pretreatment. *Renewable and Sustainable Energy Reviews, 152*, 111679. https://doi.org/10.1016/j.rser.2021.111679

Orlygsson, J. (2012). Ethanol production from biomass by a moderate thermophile, Clostridium AK1. *Icelandic Agricultural Sciences, 25*, 25–35.

Padilla-Rascón, C., Ruiz, E., Romero, I., Castro, E., Oliva, J. M., Ballesteros, I., & Manzanares, P. (2020). Valorisation of olive stone by-product for sugar production using a sequential acid/steam explosion pretreatment. *Industrial Crops and Products, 148*, 112279. https://doi.org/10.1016/j.indcrop.2020.112279

Paudel, S. R., Banjara, S. P., Choi, O. K., Park, K. Y., Kim, Y. M., & Lee, J. W. (2017). Pretreatment of agricultural biomass for anaerobic digestion: Current state and challenges. *Bioresource Technology, 245*, 1194–1205. https://doi.org/10.1016/j.biortech.2017.08.182

Paul, S., & Dutta, A. (2018). Challenges and opportunities of lignocellulosic biomass for anaerobic digestion. *Resources, Conservation and Recycling, 130*, 164–174. https://doi.org/10.1016/j.resconrec.2017.12.005

Peng, J., Xu, H., Wang, W., Kong, Y., Su, Z., & Li, B. (2021). Techno-economic analysis of bioethanol preparation process via deep eutectic solvent pretreatment. *Industrial Crops and Products, 172*, 114036. https://doi.org/10.1016/j.indcrop.2021.114036

Preethi, Gunasekaran, M., Kumar, G., Karthikeyan, O. P., Varjani, S., & Rajesh Banu, J. (2021). Lignocellulosic biomass as an optimistic feedstock for the production of biofuels as valuable energy source: Techno-economic analysis, environmental impact analysis, breakthrough and perspectives. *Environmental Technology & Innovation, 24*, 102080. https://doi.org/10.1016/j.eti.2021.102080

Procentese, A., Johnson, E., Orr, V., Garruto Campanile, A., Wood, J. A., Marzocchella, A., & Rehmann, L. (2015). Deep eutectic solvent pretreatment and subsequent saccharification of corncob. *Bioresource Technology, 192*, 31–36. https://doi.org/10.1016/j.biortech.2015.05.053

Procentese, A., Raganati, F., Olivieri, G., Russo, M. E., Rehmann, L., & Marzocchella, A. (2017). Low-energy biomass pretreatment with deep eutectic solvents for bio-butanol production. *Bioresource Technology, 243*, 464–473. https://doi.org/10.1016/j.biortech.2017.06.143

Qin, L., Zhao, X., Li, W. C., Zhu, J. Q., Liu, L., Li, B. Z., & Yuan, Y. J. (2018). Process analysis and optimization of simultaneous saccharification and co-fermentation of ethylenediamine-pretreated corn stover for ethanol production. *Biotechnology for Biofuels, 11*, 1–10. https://doi.org/10.1186/s13068-018-1118-8

Queiroz, S. S., Jofre, F. M., Mussatto, S. I., Felipe, M., & Maria das Graças, A. F. (2022). Scaling up xylitol bioproduction: Challenges to achieve a profitable bioprocess. *Renewable and Sustainable Energy Reviews, 154*, 111789. https://doi.org/10.1016/j.rser.2021.111789

Robak, K., & Balcerek, M. (2018). Review of second generation bioethanol production from residual biomass. *Food Technology and Biotechnology, 56*, 174–187. https://doi.org/10.17113/ftb.56.02.18.5428

Sadhukhan, J., Martinez-Hernandez, E., Amezcua-Allieri, M. A., Aburto, J., Honorato, S., & Aburto, J. (2019). Economic and environmental impact evaluation of various biomass feedstock for bioethanol production and correlations to lignocellulosic composition. *Bioresource Technology Reports, 7*, 100230. https://doi.org/10.1016/J.BITEB.2019.100230

Satlewal, A., Agrawal, R., Bhagia, S., Sangoro, J., & Ragauskas, A. J. (2018). Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities. *Biotechnology Advances, 36*, 2032–2050.

Scully, S. M., & Orlygsson, J. (2015). Recent advances in second generation ethanol production by thermophilic bacteria. *Energies, 8*, 1–30. https://doi.org/10.3390/en8010001

Shangdiar, S., Cheng, P. C., Chen, S. C., Amesho, K. T. T., Ponnusamy, V. K., & Lin, Y. C. (2023). Enhancing sugar yield for bioconversion of rice straw: Optimization of microwave-assisted pretreatment using dilute acid hydrolysis. *Environmental Technology and Innovation, 32*, 103313. https://doi.org/10.1016/j.eti.2023.103313

Shrotri, A., Kobayashi, H., & Fukuoka, A. (2017). Catalytic conversion of structural carbohydrates and lignin to chemicals. In *Advances in catalysis*, 1st Ed. Elsevier Inc. https://doi.org/10.1016/bs.acat.2017.09.002.

Shrotri, A., Lambert, L. K., Tanksale, A., & Beltramini, J. (2013). Mechanical depolymerisation of acidulated cellulose: Understanding the solubility of high molecular weight oligomers. *Green Chemistry, 15*, 2761–2768. https://doi.org/10.1039/c3gc40945g

Slathia, P. S., Raina, N., Kiran, A., Kour, R., Bhagat, D., & Sharma, P. (2020). Dilute acid pretreatment of pine needles of Pinus roxburghii by response surface methodology for bioethanol production by separate hydrolysis and fermentation. *Biomass Conversion and Biorefinery, 10*, 95–106. https://doi.org/10.1007/s13399-019-00433-1

Silva Ortiz, P. A., Maréchal, F., & de Oliveira Junior, S. (2020). Exergy assessment and techno-economic optimization of bioethanol production routes. *Fuel, 279*, 118327. https://doi.org/10.1016/j.fuel.2020.118327

Simangunsong, E., Ziegler-Devin, I., Chrusciel, L., Girods, P., Wistara, N. J., & Brosse, N. (2020). Steam explosion of beech wood: Effect of the particle size on the xylans recovery. *Waste and Biomass Valorization, 11*, 625–633. https://doi.org/10.1007/s12649-018-0522-4

Singh, N., Puri, M., Tuli, D. K., Gupta, R. P., Barrow, C. J., & Mathur, A. S. (2018). Bioethanol production potential of a novel thermophilic isolate Thermoanaerobacter sp. DBT-IOC-X2 isolated from Chumathang hot spring. *Biomass and Bioenergy, 116*, 122–130. https://doi.org/10.1016/j.biombioe.2018.05.009

Sitotaw, Y. W., Habtu, N. G., Gebreyohannes, A. Y., Nunes, S. P., & Van Gerven, T. (2021). Ball milling as an important pretreatment technique in lignocellulose biorefineries: A review. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-021-01800-7

Sitotaw, Y. W., Habtu, N. G., Gebreyohannes, A. Y., Nunes, S. P., & Van Gerven, T. (2023). Ball milling as an important pretreatment technique in lignocellulose biorefineries: A review. *Biomass Conversion and Biorefinery, 13*, 15593–15616. https://doi.org/10.1007/s13399-021-01800-7

Sondhi, S., Kaur, P. S., & Kaur, M. (2020). Techno-economic analysis of bioethanol production from microwave pretreated kitchen waste. *SN Applied Sciences, 2*, 1–13. https://doi.org/10.1007/s42452-020-03362-1

Sönnichsen, N. (2024). *Fuel ethanol production worldwide in 2022* [WWW Document]. Retrieved 16 January, 2024, from https://www.statista.com/statistics/281606/ethanol-production-in-selected-countries/.

Su, T., Zhao, D., Khodadadi, M., & Len, C. (2020). Lignocellulosic biomass for bioethanol: Recent advances, technology trends, and barriers to industrial development. *Current Opinion in Green and Sustainable Chemistry, 24*, 56–60. https://doi.org/10.1016/j.cogsc.2020.04.005

Talukder, M. M. R., Goh, H. Y., & Puah, S. M. (2017). Interaction of silica with cellulase and minimization of its inhibitory effect on cellulose hydrolysis. *Biochemical Engineering Journal, 118*, 91–96. https://doi.org/10.1016/j.bej.2016.11.016

Tan, Y. T., Ngoh, G. C., & Chua, A. S. M. (2019). Effect of functional groups in acid constituent of deep eutectic solvent for extraction of reactive lignin. *Bioresource Technology, 281*, 359–366. https://doi.org/10.1016/j.biortech.2019.02.010

Tan, Y. T., Ngoh, G. C., Seak, A., & Chua, M. (2018). Evaluation of fractionation and delignification efficiencies of deep eutectic solvents on oil palm empty fruit bunch. *Industrial Crops and Products, 123*, 271–277. https://doi.org/10.1016/j.indcrop.2018.06.091

Taniguchi, M., Takahashi, D., Watanabe, D., Sakai, K., Hoshino, K., Kouya, T., & Tanaka, T. (2010). Effect of steam explosion pretreatment on treatment with Pleurotus ostreatus for the enzymatic hydrolysis of rice straw. *Journal of Bioscience and Bioengineering, 110*, 449–452. https://doi.org/10.1016/j.jbiosc.2010.04.014

Tao, L., Tan, E. C. D., Aden, A., & Elander, R. T. (2013). Techno-economic analysis and life-cycle assessment of lignocellulosic biomass to sugars using various pretreatment technologies. In J. Sun, S.-Y. Ding, & J. D. Peterson (Eds.), *Biological conversion of biomass for fuels and chemicals: Explorations from natural utilizations systems* (pp. 358–380). Royal Society of Chemistry.

Thanapimmetha, A., Saisriyoot, M., Khomlaem, C., & Chisti, Y. (2019). A comparison of methods of ethanol production from sweet sorghum bagasse. *Biochemical Engineering Journal, 151*, 107352. https://doi.org/10.1016/j.bej.2019.107352

Tsegaye, B., Balomajumder, C., & Roy, P. (2019). Optimization of microwave and NaOH pretreatments of wheat straw for enhancing biofuel yield. *Energy Conversion and Management, 186*, 82–92. https://doi.org/10.1016/j.enconman.2019.02.049

Ullah, A., Zhang, Y., Liu, C., Qiao, Q., Shao, Q., & Shi, J. (2023). Process intensification strategies for green solvent mediated biomass pretreatment. *Bioresource Technology, 369*, 128394. https://doi.org/10.1016/j.biortech.2022.128394

van Rijn, R., Nieves, I. U., Shanmugam, K. T., Ingram, L. O., & Vermerris, W. (2018). Techno-economic evaluation of cellulosic ethanol production based on pilot biorefinery data: A case study of sweet sorghum bagasse processed via L+SScF. *Bioenergy Research, 11*, 414–425. https://doi.org/10.1007/S12155-018-9906-3/METRICS

Vasconcelos, M. H., Mendes, F. M., Ramos, L., Dias, M. O. S., Bonomi, A., Jesus, C. D. F., Watanabe, M. D. B., Junqueira, T. L., Milagres, A. M. F., Ferraz, A., & dos Santos, J. C. (2020). Techno-economic assessment of bioenergy and biofuel production in integrated sugarcane biorefinery: Identification of technological bottlenecks and economic feasibility of dilute acid pretreatment. *Energy, 199*, 117422. https://doi.org/10.1016/j.energy.2020.117422

Wahlström, R., Hiltunen, J., De Souza, P., Nascente Sirkka, M., Vuoti, S., & Kruus, K. (2016). Comparison of three deep eutectic solvents and 1-ethyl-3-methylimidazolium acetate in the pretreatment of lignocellulose: Effect on enzyme stability, lignocellulose digestibility and one-pot hydrolysis. *RSC Advances, 6*, 68100–68110. https://doi.org/10.1039/c6ra11719h

Wang, L., Bilal, M., Tan, C., Jiang, X., & Li, F. (2022). Industrialization progress of lignocellulosic ethanol. *Systems Microbiology and Biomanufacturing, 2*, 246–258. https://doi.org/10.1007/s43393-021-00060-w

Wang, W., & Lee, D. J. (2021). Lignocellulosic biomass pretreatment by deep eutectic solvents on lignin extraction and saccharification enhancement: A review. *Bioresource Technology, 339*, 125587. https://doi.org/10.1016/j.biortech.2021.125587

Wang, Q., Liu, S., He, C., Zhuang, X., Yu, Q., Wang, W., & Gao, Y. (2019). Recycled biorefinery system Integrating phosphoric acid/acetone pretreatment of sugarcane bagasse with subsequent platform chemicals generation. *BioResources, 14*(2), 4623–4638. https://doi.org/10.15376/biores.14.2.4623-4638

Wirawan, F., Cheng, C. L., Lo, Y. C., Chen, C. Y., Chang, J. S., Leu, S. Y., & Lee, D. J. (2020). Continuous cellulosic bioethanol co-fermentation by immobilized Zymomonas mobilis and suspended Pichia stipitis in a two-stage process. *Applied Energy, 266*, 114871. https://doi.org/10.1016/j.apenergy.2020.114871

Wu, Y., Ge, S., Xia, C., Mei, C., Kim, K. H., Cai, L., Smith, L. M., Lee, J., & Shi, S. Q. (2021). Application of intermittent ball milling to enzymatic hydrolysis for efficient conversion of lignocellulosic biomass into glucose. *Renewable and Sustainable Energy Reviews, 136*, 110442. https://doi.org/10.1016/j.rser.2020.110442

Xing, W., Xu, G., Dong, J., Han, R., & Ni, Y. (2018). Novel dihydrogen-bonding deep eutectic solvents: Pretreatment of rice straw for butanol fermentation featuring enzyme recycling and high solvent yield. *Chemical Engineering Journal, 333*, 712–720. https://doi.org/10.1016/j.cej.2017.09.176

Xu, F., Sun, J., Wehrs, M., Kim, K. H., Rau, S. S., Chan, A. M., Simmons, B. A., Mukhopadhyay, A., & Singh, S. (2018). Biocompatible choline-based deep eutectic solvents enable one-pot production of cellulosic ethanol. *ACS Sustainable Chemistry & Engineering, 6*, 8914–8919. https://doi.org/10.1021/acssuschemeng.8b01271

Yan, D., Ji, Q., Yu, X., Li, M., Abiola Fakayode, O., Yagoub, A. E. G. A., Chen, L., & Zhou, C. (2021). Multimode-ultrasound and microwave assisted natural ternary deep eutectic solvent sequential pretreatments for corn straw biomass deconstruction under mild conditions. *Ultrasonics Sonochemistry, 72*, 1–11. https://doi.org/10.1016/j.ultsonch.2020.105414

Yan, X., Wang, Z., Zhang, K., Si, M., Liu, M., Chai, L., Liu, X., & Shi, Y. (2017). Bacteria-enhanced dilute acid pretreatment of lignocellulosic biomass. *Bioresource Technology, 245*, 419–425. https://doi.org/10.1016/j.biortech.2017.08.037

Yu, Y., Long, Y., & Wu, H. (2016). Near-complete recovery of sugar monomers from cellulose and lignocellulosic biomass via a two-step process combining mechanochemical hydrolysis and dilute acid hydrolysis. *Energy and Fuels, 30*, 1571–1578. https://doi.org/10.1021/acs.energyfuels.5b02196

Zang, G., Shah, A., & Wan, C. (2020). Techno-economic analysis of an integrated biorefinery strategy based on one-pot biomass fractionation and furfural production. *Journal of Cleaner Production, 260*, 120837. https://doi.org/10.1016/j.jclepro.2020.120837

Zhang, C. W., Xia, S. Q., & Ma, P. S. (2016). Facile pretreatment of lignocellulosic biomass using deep eutectic solvents. *Bioresource Technology, 219*, 1–5. https://doi.org/10.1016/j.biortech.2016.07.026

Zhang, H., Fan, M., Li, X., Zhang, A., & Xie, J. (2018). Enhancing enzymatic hydrolysis of sugarcane bagasse by ferric chloride catalyzed organosolv pretreatment and Tween 80. *Bioresource Technology, 258*, 295–301. https://doi.org/10.1016/j.biortech.2018.03.004

Zhao, L., Zhang, X., Xu, J., Ou, X., Chang, S., & Wu, M. (2015). Techno-economic analysis of bioethanol production from lignocellulosic biomass in China: Dilute-acid pretreatment and enzymatic hydrolysis of corn stover. *Energies, 8*, 4096–4117. https://doi.org/10.3390/EN8054096

Zhu, J. Q., Li, X., Qin, L., Li, W. C., Li, H. Z., Li, B. Z., & Yuan, Y. J. (2016). In situ detoxification of dry dilute acid pretreated corn stover by co-culture of xylose-utilizing and inhibitor-tolerant Saccharomyces cerevisiae increases ethanol production. *Bioresource Technology, 218*, 380–387. https://doi.org/10.1016/j.biortech.2016.06.107

Zulkefli, S., Abdulmalek, E., Basyaruddin, M., & Rahman, A. (2017). Pretreatment of oil palm trunk in deep eutectic solvent and optimization of enzymatic hydrolysis of pretreated oil palm trunk. *Renewable Energy, 107*, 36–41. https://doi.org/10.1016/j.renene.2017.01.037