


 Cite this: *RSC Adv.*, 2022, 12, 18848

Production of butanol from lignocellulosic biomass: recent advances, challenges, and prospects

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Due to energy and environmental concerns, biobutanol is gaining increasing attention as an alternative renewable fuel owing to its desirable fuel properties. Biobutanol production from lignocellulosic biomass through acetone–butanol–ethanol (ABE) fermentation has gained much interest globally due to its sustainable supply and non-competitiveness with food, but large-scale fermentative production suffers from low product titres and poor selectivity. This review presents recent developments in lignocellulosic butanol production, including pretreatment and hydrolysis of hemicellulose and cellulose during ABE fermentation. Challenges are discussed, including low concentrations of fermentation sugars, inhibitors, detoxification, and carbon catabolite repression. Some key process improvements are also summarised to guide further research and development towards more profitable and commercially viable butanol fermentation.

 Received 28th December 2021
 Accepted 27th May 2022

DOI: 10.1039/d1ra09396g

rsc.li/rsc-advances

1. Introduction

The dual pressures of international energy security and climate change are encouraging the development of biofuels.¹ Butanol, a useful biofuel and important platform chemical, has attracted great interest due to its excellent fuel properties (high energy density, excellent mixability, low volatility and corrosiveness).^{2,3} However, high substrate cost, solvent toxicity, low butanol titre and poor productivity make butanol fermentation expensive for large-scale practical applications.⁴ Traditionally, butanol has been produced *via* acetone–butanol–ethanol (ABE) fermentation from sugars or starch as substrates, but the cost of substrates (~60% of the total cost of ABE fermentation) significantly decreased the competitiveness of ABE fermentation, so much so that biobutanol produced by *Clostridium* cannot compete with petrochemical butanol.^{5,6}

Selection of feasible substrates plays an important role in butanol production. At present, four generations of feedstocks have contributed to produce butanol, and their benefits and limitations are listed in Table 1.⁷ Among them, lignocellulosic biomass has been evaluated as cheap feedstock for biobutanol production, including rice straw,⁸ corncobs,⁹ wheat straw,¹⁰ barley straw,¹¹ sorghum bagasse,¹² and different types of wood such as

pine¹³ and elm.^{14,15} Lignocellulosic biomass is the most abundant, sustainable, and cost-effective form of biomass, hence valorisation to biofuel could provide notable benefits, including (i) CO₂ fixation from the atmosphere, (ii) reduced air pollution from incineration, and (iii) enhanced energy security for oil-importing countries.^{16,17} More importantly, utilising lignocellulosic materials could also significantly reduce environmental pollution caused by accumulation of agricultural forestry wastes,^{18–20} hence it represents an alternative strategy for large-scale biobutanol production.

Recently, several articles have been published that summarise general aspects of lignocellulosic butanol fermentation and focus on the selection of cheap feedstocks, optimisation of cost-efficient processing methods, and the development of improved microbial strains.^{21–24} Herein, we review advances in the pretreatment and hydrolysis of lignocellulosic biomass into fermentable substrates, focusing on recent research progress in overcoming the factors limiting lignocellulosic butanol fermentation. Additionally, improvement of lignocellulosic butanol fermentation by metabolic engineering and process integration strategies are also discussed. This review aims to provide guidance for improving the overall performance of lignocellulosic butanol fermentation.

2. Overall process for biobutanol production from lignocellulosic biomass

2.1 Microorganisms and metabolic pathways related to lignocellulosic butanol fermentation

Clostridia are obligate anaerobes and spore-forming bacteria with a complex life cycle, among which *C. acetobutylicum*, *C.*

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Table 1 Benefits and limitations of biomass^{21,22,25}

Feedstock	Benefits	Limitations
First-generation feedstock		
Starch and sucrose feedstocks	High butanol yield Sufficient fermentable substrates through simple pretreatment processes	Occupies potential crop land Competes with food supply Significantly increases the overall cost of fermentation
Second-generation feedstock		
Lignocellulose biomass	Cost-effective, huge carbon resources No competition with food supply	Difficult to achieve sufficient fermentable sugars from complex and recalcitrant biomass Requires complex pretreatment and detoxification processes
Third-generation feedstock		
Algal biomass	No competition with cultivation land and food supply Fewer or no lignin and fermentation inhibitors	Significantly increases the cost of downstream processes due to low production and productivity of butanol Difficult to obtain sufficient fermentable sugars
Fourth-generation feedstock		
Syngas	Increased CO ₂ capture ability No complex pretreatment process needed Directly utilises clostridia with a high production rate	Still at its infancy as a technology Several unknown key parameters that limit butanol production Poor mass transfer from gas to liquid

beijerincki, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum* are the most well-known ABE fermenters, sharing a typical life cycle and metabolic pathways for butanol production.^{26,27} Following the publication of the genomes of several solventogenic clostridia, such as *C. acetobutylicum* ATCC 824,²⁸ *C. acetobutylicum* DSM 1731²⁹ and *C. acetobutylicum* EA2018.³⁰ Clostridia are among the most important industrial production strains for biobutanol, and the main advantage is the availability of a wide range of sugar sources, including pentose (xylose and arabinose), hexose (glucose, fructose, mannose and galactose), disaccharides (lactose, sucrose, maltose and cellobiose) and polysaccharides (starch). Lignocellulosic biomass is composed of various monosaccharides, and is the most abundant renewable resource on earth. Producing biobutanol from wood cellulosic biomass with fermentable sugars an economical and effective strategy.

In ABE industrial fermentation processes, bacterial strains use sugar substrates to fuel their rapid growth, and produce acetic acid, butyric acid and other organic acids, while generating ATP (*e.g.*, the ATP yield of butyrate fermentation is 3 ATP per glucose; this is more than has been reported for fermentations to date³¹). ATP is the essential energy source needed for bacterial growth and fermentation, and this stage is the acid production stage. During acid production, organic acids such as acetic acid and butyric acid rapidly accumulate, and dissociated acids can adhere to the surface of the cell membrane as an unpaired ion, triggering a collapse of the pH gradient of the cell membrane.³² However, solvent production increases only when butyric acid accumulates and pH decreases, due to the essential role of undissociated butyric acid on the induction of solvent production in *C. acetobutylicum*.^{33,34} Subsequently, bacteria consume ATP to maintain the pH gradient across the

membrane, and synthesise acetone, ethanol and butanol, during the alcohol production stage. This stage is characterised by solvent toxicity, the most serious of which is butanol toxicity,³⁵ since the fermentation concentration is in the range of the inhibitory concentration for this solvent. To produce butanol efficiently, microbes must possess strong physiological robustness and fitness, coupled with strong metabolic capabilities, to enable them to work efficiently during bioprocesses. In some bacteria, the intracellular pH declines as a function of extracellular pH, and these are more resistant to the toxic effects of fermentation acids.³⁴ In addition, higher ATP levels can divert carbon flux toward butanol formation.³⁶

2.2 Substrates for lignocellulosic butanol

Lignocellulosic biomass, mainly comprising of cellulose, hemicellulose and lignin, are the most abundant, sustainable, and cost-effective types of biomass for butanol production.^{39,40} Among them, cellulose is an unbranched homopolysaccharide consisting of β -glucopyranosyl units that can be broken down to hexoses (C-6 sugars), and hemicellulose are branched heteropolysaccharides consisting of both hexose and pentose sugar residues.^{41,42} Lignin consists of phenylpropane units linked through different types of interunit linkages, playing a cementing role to connect cells and increasing the mechanical strength properties, which makes lignocellulosic resistant against biodegradation by microorganisms.⁴³ Therefore, removal of lignin from lignocellulose biomass before hydrolysis of cellulose and hemicellulose is highly desirable for successful lignocellulosic ABE fermentation.⁴⁴ Due to the physiological properties of solventogenic clostridia, a typical lignocellulosic butanol fermentation process can be summarised in four major steps: pretreatment (breaking down the complex structure of



lignocellulosic biomass), hydrolysis (providing fermentable monomers), fermentation and distillation^{8,45} (Fig. 1).

2.2.1 Pretreatment processes for lignocellulosic biomass.

Due to the recalcitrance of lignocellulosic biomass,⁴⁶ pretreatment is a crucial step that breaks down the complex structure of lignocellulosic materials and increases the surface area and porosity, thereby enhancing saccharification to release fermentable sugars (Fig. 2).^{47,48} Primarily, pretreatment processes can be classified into four general categories (reviewed in detail by:^{21,49} (1) physical or mechanical treatment, including milling/grinding, chipping, shredding, extrusion, microwave and ultra-sonication, which effectively breaks down the physical structure of lignocellulosic biomass;⁵⁰ (2) chemical treatment, including application of concentrated/dilute acid, alkali, ozonolysis, organic solvents and ionic liquids;^{51,52} (3) physicochemical treatment, involving the use of steam explosion, ammonium fiber explosion, CO₂ explosion and hot water, to effectively release monosaccharides and oligosaccharides;^{53,54} (4) biological treatments, using microorganisms (such as fungi, bacteria, or consortia of fungi and bacteria) to degrade lignin, hemicellulose and cellulose, potentially high selectivity of enzymatic hydrolysis,⁵⁵ but the effectiveness is influenced by substrate concentration, enzyme loading, pH and temperature.⁵⁶

There are several advantages and disadvantages for different pretreatment processes, limiting the economics and feasibility of lignocellulosic butanol. For example, dilute acid and alkali pretreatments are widely used to prepare digestible substrates,

but a neutralization step is required to maintain pH before enzymatic hydrolysis. More importantly, salts formed in the neutralisation process may also influence the efficiency of enzymatic hydrolysis and ABE fermentation.⁵⁷ Recently, deep eutectic solvents have been applied for biomass pre-treatment due to their facile preparation with 100% atom efficiency, low melting point, easy biocompatibility, cost-effectiveness, low viscosity, and environmental friendliness.^{58,59} In a short time (90 min) and with low energy consumption (120 °C) and a deep eutectic solvent (DES) pretreatment process, total sugars were increased to 37.94 g L⁻¹ (30.59 g L⁻¹ glucose, 7.35 g L⁻¹ xylose) at a low enzyme loading of 7.5 g of filter paper units (FPU) per L of pretreated corn stover (CS), and 13.65 g L⁻¹ total ABE was achieved with high productivity (0.68 g L⁻¹ h⁻¹) and yield (0.38 g g⁻¹) from DES-pretreated CS hydrolysate without any detoxification and sterilisation.⁶⁰ In addition, combinations of pretreatment method have been also employed and shown to exert synergic effects on the quality of resulting hydrolysate, including a high yield of monomeric sugars and low levels of inhibitors.^{61,62} For example, after pretreatment with optimised microwave-assisted alkali pretreatment followed by acid hydrolysis, 76.3% lignin removal, 21.1% hemicellulose and 71.9% cellulose were obtained at 640 W microwave power, 2.8% NaOH, and 19 min treatment time, producing 46.2 g L⁻¹ of reducing sugar and 18.7 g L⁻¹ ABE.⁶³

Unfortunately, such pretreatment processes are costly and time-consuming, and pretreating lignocellulosic biomass can also produce several undesirable compounds, in which the

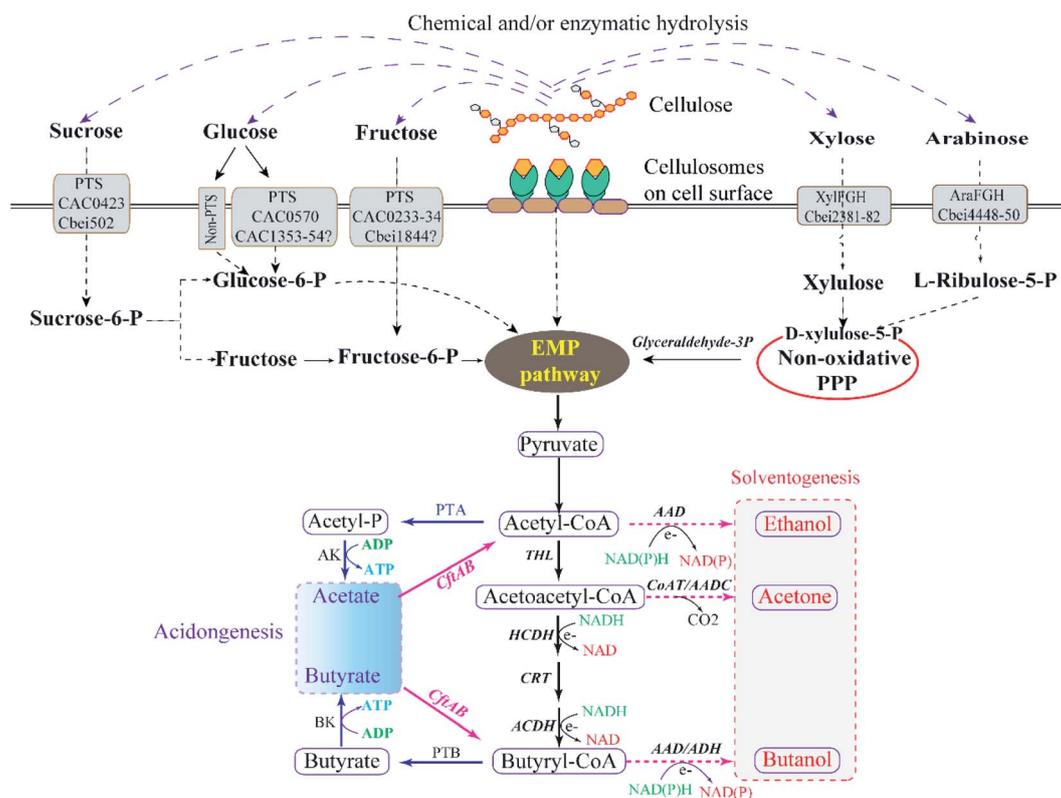


Fig. 1 Schematic diagram of butanol synthesis from lignocellulosic biomass in clostridia.^{37,38}



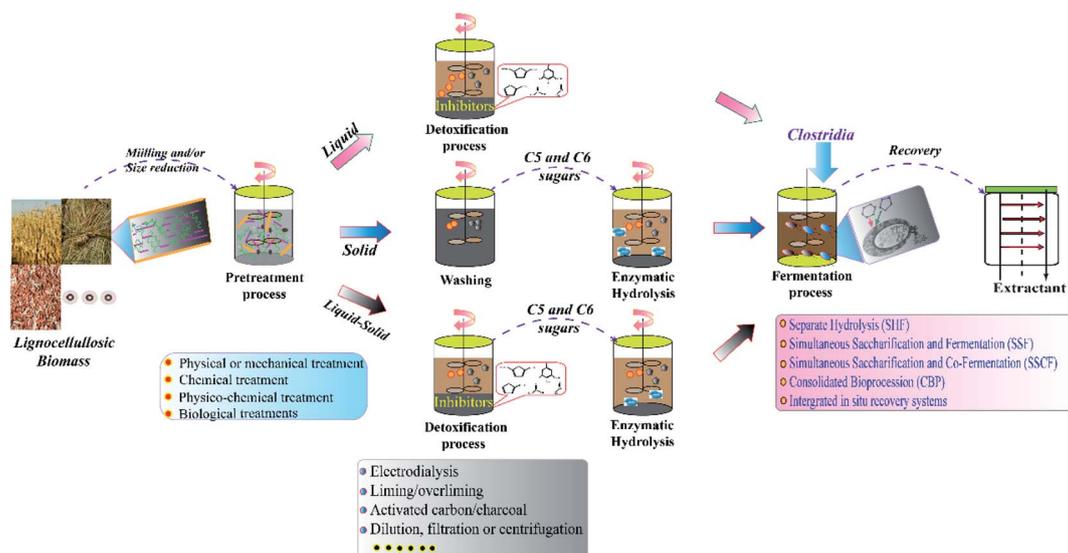


Fig. 2 Schematic diagram of fermentative butanol production from lignocellulosic biomass.^{8,44}

concentrations of inhibitors depends on the lignocellulosic biomass and pretreatment methods.¹⁷ Therefore, it is desirable to develop suitable pretreatment methods to avoid process inhibition, and thereby decrease costs and energy demands for butanol production from lignocellulosic materials.⁶⁴ Efforts could be made on the following directions: improving the conversion of lignocellulosic biomass with higher solid loading and low enzyme dosage under mild treat conditions; maximising hemicellulose and cellulose recovery with minimal degradation of lignin; or combining with microbial fermentation process optimization to obtain a more suitable substrate for butanol fermentation without increasing costs and processes. For example, lignocellulosic material wheat straw as a source of fermentable saccharides, and chicken feather as a source of amino acids and peptides, hydrolysis of both materials was carried out simultaneously, resulting in a cultivation medium that was suitable for direct use in biobutanol production.⁶⁵

2.2.2 Hydrolysis of cellulose and hemicellulose. Cellulose, accounting for ~40% of lignocellulosic materials, is a long-chain polymer formed by glucose connected by β -1,4-glycosidic bonds,⁶⁶ and it can be selectively hydrolysed to glucose by cellulolytic enzymes (e.g. *exo*-1,4- β -glucanases, *endo*-1,4- β -glucanase and β -glucosidases). The enzymatic hydrolysis efficiency of cellulose is affected by many parameters. For example, when the binding ability of cellulase is weak at high solid-phase load,⁶⁷ the surface coverage of cellulose is low, which lowers the cellulose hydrolysis efficiency. Improving the enzymatic hydrolysis efficiency of cellulose in fermentation substrates to obtain high levels of fermentable sugars to produce cellulose butanol is a major goal.⁶⁸

Hemicellulose, another cheap and abundant substrate, accounts for ~25 to 35% of lignocellulose. It is a polyphase polymer composed of several different types of mono-saccharides, the main structural units are xylose (>50%),

mannose, glucose, arabinose and galactose, and fermenting hemicellulose results in more butanol per unit mass of lignocellulose.⁴¹ Compared with the β 1 \rightarrow 4 linkages of cellulose, glycoside bonds of hemicellulose are more reactive and can be broken by chemical hydrolysis at milder conditions.⁶⁹ However, completely hydrolysing different types of glycosidic bonds requires a specific family of glycoside hydrolases, such as *endo*- β -1,4-xylanase, *endo*- β -1,4-man-nanase, α -galactosidase, and *endo*-galactanase. Therefore, although several clostridia can secrete some hemicellulases, the extracellular activity of enzymes is not adequate for direct utilisation of hemicellulose,⁷⁰ making butanol production from hemicellulose relatively inefficient due to expensive pretreatment processes.⁷¹ At present, dilute-acid hydrolysis,⁷² steam explosion,⁷³ and autohydrolysis/liquid hot water extraction,⁷⁴ have been evaluated for producing fermentable hemicellulosic sugars. For example, after treatment of hemicellulosic pre-hydrolysates by flocculation, followed by simultaneous detoxification with *Ureibacillus thermosphaericus* and *Cupriavidus taiwanensis* co-culture and hydrolysis with *Paenibacillus campinasensis*, a reduction of phenolic compounds up to 56% was achieved after flocculation, and ABE fermentation could produce 6.8 g L⁻¹ of butanol after 116 h.⁷⁵

3. Limitations and alleviation strategies for lignocellulosic butanol fermentation

Essential pretreatment and hydrolysis steps limit the fermentation of lignocellulosic biomass, accounting for a large portion of the process cost. Therefore, it is crucial to develop more cost-efficient pretreatment methods that minimise the generation of inhibitors, lower energy consumption, diminish operating costs, and simultaneously maximise fermentable sugar yields with a careful consideration of feedstock properties.⁷⁶ Due to



the low transport and utilization efficiency of fermentable sugars, low butanol yield is another major issue for lignocellulosic fermentation. Additionally, the accumulation of toxic intermediates, or overproduction of foreign proteins during butanol efficient fermentation often caused unwanted cellular stresses, resulting in a decrease in overall cell fitness. Therefore, increase of tolerance and robustness of strain is also important and necessary.^{77,78}

3.1 Lignocellulose fermentation substrate treatment

Catabolite repression, release of inhibitors, and low sugar concentrations have proved to be the limiting factors for lignocellulosic butanol fermentation, and more cost-effective processes are needed to convert hemicellulose and cellulose into fermentable hydrolysates with appropriate concentrations of sugars, inhibitors, and stimulators.

3.1.1 Low-concentration fermentable sugars. During ABE fermentation, the shift from the acidogenesis phase toward the solventogenesis phase is strongly dependent on the concentration of the carbon source after growth phase;⁷⁹ increasing the concentration of the carbon source can promote fermentation toward the solventogenic phase, and thereby increase the butanol : acetone ratio.⁸⁰ Therefore, mismatching hydrolysis and fermentation processes, in which relatively low levels of sugars can be achieved by typical enzymatic hydrolysis of cellulose, is a major obstacle for lignocellulosic butanol production.⁶⁸ Achieving high concentrations of fermentable sugars is crucial for lignocellulosic butanol, and several strategies have been developed to increase the total sugar concentration from treatment process:⁸¹ (1) using a fractionation pretreatment (*e.g.*, organosolv pretreatment) to generate a cellulose-rich pretreated solid; (2) preparation of hydrolysates by simultaneous enzymatic hydrolysis of cellulose and hemicellulose,⁸² or supplementing with starch for ABE fermentation,⁸³ but carbon catabolite repression (CCR) may be an issue;⁸⁴ (3) concentrating hydrolysates by filtration or evaporation, increasing the total sugar concentration to 30–80 g L⁻¹.⁸⁵

3.1.2 Inhibitors and detoxification processes

3.1.2.1 Lignocellulose-derived inhibitors. During pretreatment of lignocellulose, several lignocellulose-derived microbial inhibitory compounds (LDMICs)⁸⁶ are often generated, in which the amounts and species of inhibitors are dependent on raw materials and/or pretreatment processes.⁵⁴ These inhibitors can be classified into weak organic acids, furan derivatives, and phenolics, with toxicity at the same dose against ABE production ranked formic acid > phenols > furfurals.⁸⁷ More interestingly, clostridia have different tolerances of and stress responses to these inhibitors.⁸⁸ Compared with *C. beijerinckii*,⁸⁹ *C. acetobutylicum* is more sensitive to formic acid, and 0.1 M formic acid induces an 'acid crash' that completely inhibits butanol production and cell growth.⁹⁰ However, low concentrations (0.5–2 g L⁻¹) of furfural and 5-hydroxymethyl furfural can stimulate *Cupriavidus basilensis*, *C. beijerinckii* BA101,⁹¹ *C. beijerinckii* P260 (ref. 92) and *C. acetobutylicum* ATCC 824.⁹³

3.1.2.2 Lignin-derived compounds. Compared with weak organic acids and furan derivatives, partial decomposition of

(poly)phenolic aromatic compounds in lignin, such as syringaldehyde, vanillic acid and ferulic acid,⁹⁴ is more toxic to clostridia.^{95–97} More importantly, combinations of acids and furan aldehydes, as well as different phenolics, can have synergistic effects on ABE fermentation,⁹⁸ but the sophisticated physiological regulation mechanism remains unclear.⁹⁹ For example, during ABE fermentation, production of butanol and total solvent was decreased to 6.07 g L⁻¹ from 8.08 g L⁻¹, and to 11.71 g L⁻¹ from 13.95 g L⁻¹, respectively, under 0.5 g L⁻¹ of ferulic acid stress,¹⁰⁰ and the biosynthesis of organic acids was severely inhibited at lower concentrations of phenolics (0.2 g L⁻¹ vanillin or/and vanillic acid).⁹⁸ Furthermore, lignosulfonates, lignin-based organic polymers, can also significantly downregulate glycolysis and the butanol biosynthetic pathway in clostridia due to the unique properties of these anionic, water-soluble, highly acidic compounds.¹⁰¹ For example, augmenting the lignosulfonate concentration >0.5 g L⁻¹ led to a significant decrease in solvent titre (ABE ~1.50 g L⁻¹), while >1 g L⁻¹ of low-molecular-weight lignosulfonate seriously inhibited solvent synthesis, and even completely blocked the process of ABE fermentation.¹⁰²

3.1.2.3 Hemicellulose-derived inhibitors. Hexoses and pentoses could be obtained *via* the chemical hydrolysis of hemicellulose, however, often accompanying with the formation of 5-hydroxymethyl furfural (HMF) and furfural due to dehydration reactions.^{103,104} More importantly, furan compounds can be also subjected to rehydration reactions to form levulinic acid and formic acid under acidic conditions, providing inhibitory effect on *C. acetobutylicum* due to the acid crash.^{67,90} In addition, acetic acid is also produced through hydrolysis of the acetyl group of hemicellulose, and 10 g L⁻¹ of acetic acid slightly increases the ABE fermentation performance, but the higher levels significantly inhibit the fermentation performance of *C. acetobutylicum*.⁸⁹ Furthermore, the presence of monosaccharides, oligomers, and some ingredients of hemicellulosic hydrolysates can also inhibit cellulase activity, and then negatively influence the hydrolysis efficiency of cellulose.¹⁰⁵

3.1.2.4 Strategies to alleviate inhibition. In general, levels of inhibitors and fermentable sugars in hydrolysates are dependent on the feedstock and the conditions of pretreatment and hydrolysis processes.¹⁰⁶ Thus, several strategies have been developed to counteract inhibition problems, including (1) selecting less recalcitrant feedstocks; (2) utilising mild pretreatment conditions; (3) biological hydrolysis of hemicelluloses with specific enzymes, or co-culture of microorganisms producing hydrolysing enzymes¹⁰⁷ to effectively decrease the formation of inhibitors (but this can increase several incumbent costs);¹⁰⁸ (4) combining pretreatment, hydrolysis, fermentation and product recovery into a single consolidated process;¹⁰⁹ (5) integrating several detoxification methods, such as electro dialysis,¹¹⁰ liming/overlimiting,¹¹¹ activated carbon/charcoal,¹¹² *in situ* extraction with oleyl alcohol,¹¹³ and filtration or centrifugation, and resin treatments¹¹⁴ to remove solids and undissolved lignin in lignocellulosic hydrolysates. However, although these inhibitors can be effectively removed during the detoxification process,¹¹⁵ partial fermentable sugars are also



removed from lignocellulosic hydrolysates,¹¹⁶ making the detoxification process impractical for commercial application.¹¹⁷ Thus, it is necessary to develop a cost- and energy-efficient pretreatment stage to improve the conversion of lignocellulosic biomass with higher solid loading and low enzyme dosage under mild treat conditions, thereby maximising hemicellulose and cellulose recovery with minimal degradation of lignin, or minimise inhibitor formation to allow fermentation without detoxification.⁶⁵

3.2 Transport and utilization efficiency of fermented sugars

The transportation and metabolic pathways of substrates during butanol synthesis have been dissected alongside stoichiometric glucose reactions.^{30,118} Clostridia use PEP (phosphoenolpyruvate)-dependent PTSs (phosphotransferase systems) and/or non-PTS mechanisms to assimilate glucose during ABE fermentation, and PTSs are predominant in the solventogenic phase. However, some clostridia are dominated by non-PTSs (ATP-dependent glucokinases) during the solventogenic stage, such as the butanol-hyperproducing mutant strain *C. beijerinckii* BA101, derived from NCIMB 8052.¹¹⁹ PTSs transport and phosphorylate sugar substrates, play a central role in metabolic regulation. For example, there are 13 PTSs in *Clostridium* related to acetone and butanol,¹²⁰ among which 12 are encoded by chromosomal genes and can be used to transport glucose, mannose, fructose, lactose, cellulose-disaccharide, sucrose, galactose and maltose. The other PTS is encoded by genes on the large PSOL1 plasmid, and is used for transporting mannose and fructose. Some PTSs can transport multiple sugars simultaneously.¹²¹ The uptake of sugars such as pentose proceeds through non-PTS pathway; xylose and arabinose enter the cell through their specific transporters, then converge into xylose-5-phosphate through isomerisation and phosphorylation, respectively, and enter central metabolism through PPP (pentose phosphate pathway) or PK (pyruvate kinase) pathways.¹²²

Apart from dissimilarities in uptake systems utilised for sugars, carbon catabolic repression can also lead to the consumption of sugars at different rates.¹²³ After hydrolysis of lignocellulosic biomass, various ratios of fermentable sugars containing a mixture of pentoses and hexoses are released into the culture broth. Unfortunately, wild-type clostridia are poor at consuming mixed fermentable sugars due to CCR, and the utilisation of pentose sugars can be decreased or inhibited markedly in the presence of glucose.⁸⁴ Therefore, efficient utilisation of mixtures of sugars has become a prerequisite for effective lignocellulosic butanol fermentation. To alleviate CCR in mixed sugar fermentation systems, several valuable strategies have been employed, including engineering metabolic pathways (such as knocking out *ccpA* to facilitate xylose and glucose utilisation¹²⁴), mixed culture consolidated bioprocess,¹²⁵ exogenous supplementation with trace elements¹²⁶ and semi-hydrolysis strategies.¹²⁷ For example, the synergistic effect of calcium and zinc was investigated in ABE fermentation by *C. acetobutylicum* using glucose, xylose and glucose/xylose mixtures as carbon sources. As a result, glucose/xylose

utilisation, cell growth, acid re-assimilation and butanol biosynthesis were significantly improved, with butanol and ABE production increased from 11.7 and 19.4 g L⁻¹ to 16.1 and 25.9 g L⁻¹, respectively, from 69.3 g L⁻¹ glucose.¹²⁸ Similarly, semi-hydrolysates with low enzyme loading using H₂SO₄-pretreated rice straw was also employed to further improve butanol fermentation efficiency through preferential production of cellobiose and xylose (instead of glucose). As a result, butanol productivity was correspondingly increased from 0.0628 g L⁻¹ h⁻¹ to 0.265 g L⁻¹ h⁻¹ during fermentation of undetoxified semi-hydrolysates with a high cell density.¹²⁷

Therefore, efficient transport and utilization of the available sugars in lignocellulosic hydrolysate without causing or alleviating the glucose carbon repression effect could be a potential exploring direction to improve the efficiency of lignocellulosic butanol production in the future.

3.3 Fermentation strain with good physiological characteristics

There are various stress responses under varying environment stresses in the process of butanol production using industrialized fermentation of lignocellulose, such as various inhibitors in the fermentation matrix after cellulose pretreatment and hydrolysis,¹²⁹ intermediate metabolites (organic acids such as butyric acid and acetic acid accumulate) and fermentation end-products (accumulation of solvents such as butanol, ethanol, and acetone) during ABE fermentation,¹³⁰ strains exposed to physical pressures such as inappropriate pH, temperature, dissolved oxygen and osmotic pressure due to the inhomogeneity of large-scale fermentation system, and overproduction of exogenous proteins caused by various modifications or accumulation of toxic intermediates for improving the fermentation yield or output.⁷⁸ In light of it, ideal strains for butanol production by lignocellulose possess should physiological functions such as favorable adaptability and robustness for maintaining highly-active metabolic flux with good substrate and environmental tolerance of butanol during industrial fermentation.

4. Strategy for improving lignocellulosic butanol production

4.1 Improving lignocellulosic fermentation using microbial consortia or utilizing microorganisms cooperate

In lignocellulosic butanol fermentation, the complexity of the lignocellulose degradation process and the butanol metabolic pathway can significantly burden the metabolism of single strains, resulting in low butanol production using monocultures. In nature, lignocellulosic biomass can be entirely degraded and assimilated by microbial consortia containing fungi and cellulose-degrading bacteria.¹³¹ In this regard, natural microbial communities were mimicked to construct a synthetic 'Y-shaped' consortium consisting of two strains with the same butanol biosynthetic pathway and orthogonal capacity for glucose and xylose metabolism. The resulting consortium could not only adapt to environmental perturbations, but also



simultaneously utilise C5/C6 sugars in different ratios, with the most efficient butanol production from mixed sugars through equally efficient orthogonal consumption of C5 and C6 sugars.¹³² Similarly, cellulosic butanol could be produced using anaerobic co-cultures of *C. saccharoperbutylacetonicum* and white-rot fungus *Phlebia* sp. MG-60-P2. Following knockout of pyruvate decarboxylase, co-cultures of KO77 and *C. saccharoperbutylacetonicum* could displayed synergistically enhanced saccharification and butanol production was further increased from 2.5 g L⁻¹ to 3.2 g L⁻¹ compared with co-cultures of MG-60-P2 and *C. saccharoperbutylacetonicum*.¹³³ Therefore, constructing microbial consortia with the capacity for butanol synthesis and degrading lignocellulosic biomass has potential for improving lignocellulosic butanol production.

4.2 Application of advanced fermentation strategies

Following physical, chemical or biological pretreatment processes, four fermentation processes, hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and the processes consolidated bioprocessing (CBP), have been applied to overcome the complex characteristics of feedstocks and feedback inhibition for lignocellulosic butanol production (Table 2).^{134,135}

4.2.1 Simultaneous saccharification and fermentation (SSF). Compared with SHF, simultaneous saccharification and fermentation (SSF) has fewer processing steps, lower operating costs, less contamination risk, and lower feedback inhibition of sugars on enzymes, which improves the efficiency of hydrolysis and fermentation, resulting in a higher yield of butanol.⁶⁸ However, the SSF mode of operation is challenging in a number of ways: (1) during the SSF process, matching the optimum temperatures between enzymolysis (45–50 °C) and ABE fermentation (35–37 °C) is a major hurdle to achieving high cellulase activity, sugar yield, and butanol production.^{136,137} To this end, *C. acetobutylicum* with improved thermotolerance at 39–45 °C was engineered and used in high-temperature SSF of pretreated corn stover, and the optimised SSF process at 42 °C with a 12 h pre-hydrolysis yielded 10.8 g L⁻¹ of butanol and 18.2 g L⁻¹ of ABE from overlimited acid-treated liquid;¹³⁸ (2) an imbalance in the rates of sugar formation by enzymatic hydrolysis and sugar consumption by clostridia is another challenge.¹³⁹ In this regard, water-soluble cellulose oligomers have been used to modify the SSF process, in which chemically prepared water-soluble oligomers and pretreated cellulose were simultaneously subjected to enzymatic hydrolysis and ABE fermentation in a process named simultaneous co-saccharification and fermentation (SCSF).¹⁴⁰ The SCSF process achieved a higher yield of butanol/lignocellulosic biomass (g/g), and is suited for pretreatment and enzymatic hydrolysis using lignocellulosic biomass as the substrate.¹⁴¹ Recently, a novel fed-batch SSFR (simultaneous saccharification, fermentation and recovery) strategy was developed and applied for ABE fermentation from alkaline-pretreated rice straw. After optimisation of initial solids and enzyme loadings by batch SSF assays, the maximum butanol concentration and butanol productivity

were increased to 24.80 g L⁻¹ and 0.344 g L⁻¹ h⁻¹, respectively, through three biomass feedings, showing that SSFR can significantly enhance the performance of lignocellulose fermentation when accompanied by efficient enzyme use.¹⁴²

4.2.2 Consolidated bioprocesses. Recently, CBP that integrates cellulase production, lignocellulose hydrolysis, and fermentation of mixtures of sugars into a single consecutive process with a single microbe or a microbial consortium has been developed for lignocellulosic fermentation.^{143,144} For lignocellulosic butanol fermentation, several CBP processes have been carried out either by genetically engineering strains^{145–147} or co-culturing biofuel-producing strains with saccharolytic strains such as *C. cellulolyticum*¹⁴⁸ and *C. thermocellum*.¹⁴⁹ For example, a microbial consortium combining *Thermoanaerobacterium thermosaccharolyticum* M5 and *C. acetobutylicum* NJ4 was constructed for efficient ABE fermentation using xylan *via* CBP. In this study, strain M5 could efficiently degrade xylan, accumulating 19.73 g L⁻¹ of xylose within 50 h, and strain NJ4 could efficiently utilise xylose to effectively decrease substrate inhibition on xylanase and xylosidase. As a result, the synergy of the two strains could produce 13.28 g L⁻¹ of butanol from 70 g L⁻¹ of xylan under optimal conditions.¹⁵⁰

4.2.3 Two-stage and multi-stage fermentation. Compared with batch fermentation, single-stage continuous fermentation can achieve high solvent productivity, but lower solvent concentrations, indicating that single-stage continuous fermentation is not suitable for industrial-scale applications.¹⁵¹ However, a continuous bioreactor with immobilised cells and multistage fermentation has been applied for continuous ABE fermentation.¹⁵² Two-stage and multi-stage fermentation systems can completely utilise substrates and effectively assimilate acids to solvents, achieving increased productivity and solvent concentrations over long-term fermentation.¹⁵² For example, a four-stage continuous fermentation with cane molasses was employed to enhance butanol titre and productivity, achieving 13.75 g L⁻¹ of solvents with a productivity of 0.439 g L⁻¹ h⁻¹ with a gradient dilution mode of 0.15–0.15–0.125–0.1 h⁻¹.¹⁵³ In addition, a novel two-stage fermentation process, comprising an acidogenic fermentation process followed by an ABE fermentation process, was also developed and introduced to maximise sugar utilisation and butanol yield with alkaline-pretreated rice straw.¹⁵⁴ A sugar-rich hydrolysate (90.4 g L⁻¹ reducing sugar) and a high acid level in the fermentation broth (33.9 g L⁻¹ butyric acid) were obtained. Also, final butanol production was significantly increased to 15.9 g L⁻¹ with 3-fold lower cellulase loading, yielding 149 g butanol and 36 L hydrogen gas from 1 kg rice straw, respectively.¹⁵⁴

4.2.4 Improving fermentation processes for lignocellulosic butanol. In general, pretreatment processes have three main drawbacks:¹⁵⁵ (1) requiring specific instruments and facilities to pretreat lignocellulosic materials under strict conditions; (2) the formation and release of inhibitors that negatively impact enzymatic hydrolysis and fermentation, resulting in the need for an additional detoxification step, which increases total costs;¹⁵⁶ (3) using 'non-green' chemicals as catalysts in several pretreatment methods, with consequent negative environmental impacts.¹⁵⁷ To overcome these drawbacks, a 'semi-



Table 2 Summary of pretreatment and detoxification techniques applied to lignocellulosic materials for ABE fermentation^a

	Pretreatment and/hydrolysis	Detoxification	Organism	Fermentation conditions	ABE, g L ⁻¹	Butanol, g L ⁻¹	Ref
Rice straw	Acid hydrolysis + shear stress	Filtering with a sterile cotton cloth	<i>C. acetobutylicum</i> NCIM233	Batch fermentation in 2 L bioreactor	20.56	13.5	162
Corn cob	NaOH + enzymatic hydrolysis	NA	<i>C. saccharobutylicum</i> DSM 13864	Batch fermentation	19.44	12.27	163
Corn straw	Acid pre-impregnated steam explosion (APSE) pretreatment	Concentrating with Rotavapor; and then lime-treated hydrolysate with Ca(OH) ₂	<i>C. acetobutylicum</i> ATCC 824	Batch fermentation	0.31 g g ⁻¹ sugar	0.18 g g ⁻¹ sugar	164
Switchgrass	Alkali + enzyme	NA	<i>C. saccharobutylicum</i> DSM13864	Batch fermentation	22.7	13	165
Switchgrass	Thermal hydrolysis pretreatment	Without detoxification process	<i>C. beijerinckii</i> ATCC 51743	Batch fermentations	18.5	NA	166
Wheat straw	Biological treatment	NA	Coculture of <i>C. beijerinckii</i> 10132 with <i>C. cellulovorans</i> 35296	Batch fermentation	23.30	14.20	167
Wheat straw	Ammonium sulfite pretreatment	NA	<i>C. acetobutylicum</i> ATCC 824	Simultaneous saccharification and fermentation	19.83	12.64	168
Barley straw hydrolysate	Mechanical + acidic pretreatment	Overliming method	<i>C. beijerinckii</i> P260	Fermentation with product removal by gas stripping	47.20	30.86	169
Mango peel waste	Mechanical + enzyme hydrolysis	NA	<i>C. acetobutylicum</i> NCIM 2878	Batch fermentation	15.13	10.50	170
Apple peel waste	Mechanical	NA	<i>C. acetobutylicum</i> DSM 792	Batch fermentation	20.00	14.00	171
Apple pomace	Acid pretreatment + enzymatic hydrolysis	NA	<i>C. beijerinckii</i> P260	Batch fermentation	10.8	5.6	19
Sago waste	Acid hydrolysis + enzyme hydrolysis	NA	<i>Clostridium beijerinckans</i> and <i>Bacillus coagulans</i>	Batch fermentation	9.01	3.36	172
Wheat red dog	Enzymatic digests	NA	<i>C. beijerinckii</i> NCIMB 8052	Batch fermentation	20.0	15.0	173
Spent sulfite liquor (SSL)	NA	Coupling nanofiltration, ultrafiltration and ion exchange	<i>C. acetobutylicum</i> ATCC 824	Submerged fermentation	21.09	12.96	174
Cellulosic material	NA	NA	White-rot fungus <i>Phlebia</i> sp. MG-60-P2 and <i>C. saccharoperbutylacetonicum</i>	Anaerobic co-culture in consolidated bioprocessing	NA	3.2	133
Sugarcane straw	Microwave-assisted alkali pretreatment + acid hydrolysis	NA	<i>C. beijerinckii</i> NBRC 109359	Batch fermentation	18.7	NA	63
Industrial vinegar residue	Steam explosion pretreatment + enzymatic hydrolysis	NA	<i>C. acetobutylicum</i>	Batch fermentation	12.59	7.98	175
Food waste	Liquefied and saccharified	NA	<i>C. saccharoperbutylacetonicum</i>	Continuous immobilized-cell fermentation system at dilution rate of 0.1 h ⁻¹	19.65	NA	176
Yellow top (<i>Physaria fendleri</i>)	Dilute acid + enzymatic hydrolysis	NA	<i>C. beijerinckii</i> P260	Batch fermentation	28.8	NA	177

Table 2 (Contd.)

	Pretreatment and/hydrolysis	Detoxification	Organism	Fermentation conditions	ABE, g L ⁻¹	Butanol, g L ⁻¹	Ref
Banana crop residue	Dilute alkali and acid + enzymatic saccharification	NA	<i>C. beijerinckii</i>	Batch fermentation	20.5	14.0	178
Sugarcane bagasse	Hemicellulosic hydrolysate	Without detoxification process	<i>C. saccharoperbutylacetonicum</i>	Batch fermentation	7.11	5.85	179
Hardwood hemicelluloses	Hydrolysate + enzyme	Coagulation–floculation + inhibitors removal with bacteria	<i>C. acetobutylicum</i> ATCC 824	Batch fermentation	11.8	6.8	75
Paper pulp	Enzymatic hydrolysis	NA	<i>C. saccharoperbutylacetonicum</i> ATCC 13564	Simultaneous saccharification and fermentation with <i>in situ</i> butanol recovery	NA	19.2–22.0	158

^a NA, data not available.

hydrolysis' strategy was proposed to utilise lignocellulosic material containing oligosaccharides and monosaccharides using less hydrolytic enzyme, then further improving the butanol yield, enzyme loading, and overall butanol productivity.¹²⁷ For example, a consolidated fermentation system was established in which enzymatic semi-hydrolysis of paper pulp (93.2% glucan) without pretreatment and with low enzyme loading produced high levels of cellobiose (13.9 g L⁻¹) and glucose (21.3 g L⁻¹) *via* ABE fermentation, which was as efficient as fermentation using commercial sugars without inhibitors.¹⁵⁸

Furthermore, physical, chemical or biological detoxification methods have been developed for eliminating toxic inhibitors, but the resulting wastewater, increased energy costs, and sugar loss remain barriers to economic viability and environmental sustainability at industrial scale.^{17,159} Therefore, mutagenesis, genetic manipulation and metabolic perturbations have been applied to construct inhibitor-tolerating strains that can achieve lignocellulosic butanol fermentation without detoxification or wastewater generation. For example, a mutant over-expressing PTS^{GlcG} exhibited improved inhibitor tolerance, and produced 10.1 g L⁻¹ of butanol using corn stover hydrolysate (CSH) culture without detoxification, an increase by 300% and 400% compared with 2.5 and 2.0 g L⁻¹ achieved control and PTS^{GlcG}-deficient strains, respectively.¹⁶⁰ Similarly, introduction of a 'push–pull' strategy in *C. cellulovorans* DSM 743B diverted carbon flux from acetyl-CoA to butyryl-CoA by overexpressing a *trans*-enoyl-coenzyme A reductase gene (*ter*), and an acid re-assimilation pathway uncoupled from acetone synthesis was also constructed to redirect carbon flux from butyrate and acetate toward butyryl-CoA. Xylose metabolism was engineered by overexpressing xylT (CA_C1345) and inactivating xylR (Clocel_0594) and araR (Clocel_1253), and final production of butanol was increased to 4.96 g L⁻¹ directly from alkali-extracted corncobs.¹⁶¹

4.3 A strategy for engineering physiological functionalities

Good physiological performance of microbes is crucial for successful biological fermentation. Strain improvement through physiological engineering relies on integration of knowledge on physiological functionality and efficient engineering approaches.¹³⁰ For example, introduction of pro (precursor region)–mtg (microbial transglutaminase) into *C. acetobutylicum* improves oxidative-stress resistance, growth performance, and solvent production of the host.¹⁸⁰ In the past, many strains obtained by metabolic engineering strategies failed to steady exhibit the expected phenotype because the physiological stress responses of microorganisms were ignored. Therefore, microorganisms should not only have strong metabolic capacity, but also possess strong physiological robustness and adaptability in order to work effectively in actual biological processes.

5. Future prospects

Clostridial species generally perform ABE fermentation using the cellulose and hemicellulose fraction of lignocellulosic



feedstocks.¹⁸¹ However, essential pretreatment steps limit the fermentation of lignocellulosic biomass, accounting for a large portion of the process cost. Therefore, it is crucial to develop more cost-efficient pretreatment processes that minimise the formation of inhibitors, lower energy consumption, diminish operating costs, and concurrently maximise fermentable sugar production with careful consideration of feedstock properties.⁷⁶ Carbon loss can occur in the form of xylose during biomass pretreatment, and a large amount of carbon in the form of CO₂ is emitted during fermentation and co-generation processes.^{182,183} An innovative way to valorise off-gas streams is to produce alcohols through gas fermentation, in which acetogens ferment CO₂ plus H₂ to produce VFAs (volatile fatty acids) and alcohols *via* the Wood-Ljungdahl pathway, as demonstrated with *Clostridium carboxidivorans*.^{184–186} Furthermore, effective utilisation of xylose in hemicellulose is also a key factor to reduce the production cost of biobutanol. To increase the overall competitiveness of lignocellulosic butanol, cost-effective and industrially feasible strategies for lignocellulosic biomass pretreatment and hydrolysis must be further optimised. Furthermore, an ideal lignocellulosic butanol-producing strain is also needed to maintain high butanol metabolic flux activity, efficient sugar transport, the use of fermentable mixed sugar, and good substrate tolerance in the process of industrial butanol fermentation, which can further improve the fermentation performance and lower fermentation costs.

The performance of lignocellulosic fermentation could be improved as follows:

- Process integration and intensification measures to further improve the performance and economic competitiveness of lignocellulosic butanol fermentation, such as integration of (1) pretreatment with hydrolysis, (2) hydrolysis with fermentation, and even (3) hydrolysis, fermentation, and product recovery.

- Adapting and improving the physiological characteristics of target microorganisms: it can achieve rapid transformation of hexose and pentose; good tolerance to the target product butanol, the intermediate product butyric acid, and inhibitors in raw materials; under low pH stress conditions, and in the presence of organic acids and butanol, the metabolic activity can remain high; good metabolic flux can be maintained throughout the fermentation process.

- Production of butanol by lignocellulose is so complicated that might lead to unnecessary cell stress. In that case, the toxicity problem of the butanol fermentation process and the heterogeneity of industrial production should be considered although it is essential to orient at breaking through a single problem. More precisely, lignocellulose pretreatment, microbial physiological process and characteristics, carbon efficient utilization or butanol recovery technology should be adopted to improve the physiologic performance of microbes, achieving both the enhanced yield and stable output in conjunction with interleaving functions of biological robustness.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

This research was financially supported by the Key Project of Guangxi Scientific Research and Technology Development Plan (AB17190534), the Natural Science Foundation of Guangxi (GXNSFAA259007), and the Basic Operating Expenses of Guangxi Academy of Sciences (2019YJJ1001).

References

- 1 A. C. dos Santos, *et al.*, Lignin-Enzyme Interactions in the Hydrolysis of Lignocellulosic Biomass, *Trends Biotechnol.*, 2019, 37(5), 518–531.
- 2 F. Cebreiros, M. D. Ferrari and C. Lareo, Cellulose hydrolysis and IBE fermentation of eucalyptus sawdust for enhanced biobutanol production by *Clostridium beijerinckii* DSM 6423, *Ind. Crops Prod.*, 2019, 134, 50–61.
- 3 Y. J. Jiang, *et al.*, *Clostridium* sp. Strain NJ4: A Promising Solventogenic Strain for Butanol Production from Jerusalem Artichoke through Consolidated Bioprocessing, *Energy Fuels*, 2020, 34(3), 3406–3411.
- 4 Y. Jiang, *et al.*, Current status and prospects of industrial bio-production of *n*-butanol in China, *Biotechnol. Adv.*, 2015, 33(7), 1493–1501.
- 5 N. K. N. Al-Shorgani, *et al.*, Continuous butanol fermentation of dilute acid-pretreated de-oiled rice bran by *Clostridium acetobutylicum* YM1, *Sci. Rep.*, 2019, 9, 4622.
- 6 C. Xue, *et al.*, Recent advances and state-of-the-art strategies in strain and process engineering for biobutanol production by *Clostridium acetobutylicum*, *Biotechnol. Adv.*, 2017, 35(2), 310–322.
- 7 M. Rastogi and S. Shrivastava, Recent advances in second generation bioethanol production: An insight to pretreatment, saccharification and fermentation processes, *Renewable Sustainable Energy Rev.*, 2017, 80, 330–340.
- 8 H. Amiri, K. Karimi and H. Zilouei, Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production, *Bioresour. Technol.*, 2014, 152, 450–456.
- 9 J. Zhang, *et al.*, Efficient acetone-butanol-ethanol production from corncob with a new pretreatment technology-wet disk milling, *Bioenergy Res.*, 2013, 6(1), 35–43.
- 10 N. Qureshi, B. C. Saha and M. A. Cotta, Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*, *Bioprocess Biosyst. Eng.*, 2007, 30(6), 419–427.
- 11 N. Qureshi, *et al.*, Production of butanol (a biofuel) from agricultural residues: Part I - Use of barley straw hydrolysate, *Biomass Bioenergy*, 2010, 34(4), 559–565.
- 12 Y. Jafari, K. Karimi and H. Amiri, Efficient bioconversion of whole sweet sorghum plant to acetone, butanol, and ethanol improved by acetone delignification, *J. Cleaner Prod.*, 2017, 166, 1428–1437.
- 13 H. Amiri and K. Karimi, Autohydrolysis: A promising pretreatment for the improvement of acetone, butanol,



- and ethanol production from woody materials, *Chem. Eng. Sci.*, 2015, **137**, 722–729.
- 14 A. Abedini, H. Amiri and K. Karimi, Efficient biobutanol production from potato peel wastes by separate and simultaneous inhibitors removal and pretreatment, *Renewable Energy*, 2020, **160**, 269–277.
 - 15 R. R. Mishra, *et al.*, Process optimization for conversion of waste banana peels to biobutanol by a yeast co-culture fermentation system, *Renewable Energy*, 2020, **162**, 478–488.
 - 16 K. M. Schwarz, *et al.*, Towards improved butanol production through targeted genetic modification of *Clostridium pasteurianum*, *Metab. Eng.*, 2017, **40**, 124–137.
 - 17 N. R. Baral and A. Shah, Microbial inhibitors: formation and effects on acetone-butanol-ethanol fermentation of lignocellulosic biomass, *Appl. Microbiol. Biotechnol.*, 2014, **98**(22), 9151–9172.
 - 18 C. Xlab, *et al.*, High-efficient cellulosic butanol production from deep eutectic solvent pretreated corn stover without detoxification, *Ind. Crops Prod.*, 2021, **162**, DOI: [10.1016/j.indcrop.2021.113258](https://doi.org/10.1016/j.indcrop.2021.113258).
 - 19 Q. Jin, *et al.*, Acetone-butanol-ethanol (ABE) fermentation of soluble and hydrolyzed sugars in apple pomace by *Clostridium beijerinckii* P260, *Fuel*, 2019, **244**, 536–544.
 - 20 B. Pratto, *et al.*, Biobutanol production from sugarcane straw: Defining optimal biomass loading for improved ABE fermentation, *Ind. Crops Prod.*, 2020, **148**, DOI: [10.1016/j.indcrop.2020.112265](https://doi.org/10.1016/j.indcrop.2020.112265).
 - 21 L. D. Gottumukkala, K. Haigh and J. Gorgens, Trends and advances in conversion of lignocellulosic biomass to biobutanol: Microbes, bioprocesses and industrial viability, *Renewable Sustainable Energy Rev.*, 2017, **76**, 963–973.
 - 22 Y. Wang, *et al.*, Current advances on fermentative biobutanol production using third generation feedstock, *Biotechnol. Adv.*, 2017, **35**(8), 1049–1059.
 - 23 Y. Yang, *et al.*, Metabolic regulation in solventogenic *Clostridia*: regulators, mechanisms and engineering, *Biotechnol. Adv.*, 2018, **36**(4), 905–914.
 - 24 B. Bharathiraja, J. Jayamuthunagai, T. Sudharsanaa, A. Bharghavi, R. Praveenkumar, M. Chakravarthy, D. Yuvaraj, *et al.*, Biobutanol - An impending biofuel for future: A review on upstream and downstream processing techniques, *Renewable Sustainable Energy Rev.*, 2017, **68**(1), 788–807.
 - 25 K. Dutta, A. Daverey and J. G. Lin, Evolution retrospective for alternative fuels: First to fourth generation, *Renewable Energy*, 2014, **69**, 114–122.
 - 26 Y. N. Zheng, *et al.*, Problems with the microbial production of butanol, *J. Ind. Microbiol. Biotechnol.*, 2009, **36**(9), 1127–1138.
 - 27 P. Dürre, *et al.*, Fermentative butanol production: bulk chemical and biofuel, *Ann. N. Y. Acad. Sci.*, 2008, **1125**(1), 353.
 - 28 J. Nölling, *et al.*, Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*, *J. Bacteriol.*, 2001, **183**(16), 4823–4838.
 - 29 G. H. Bao, *et al.*, Complete Genome Sequence of *Clostridium acetobutylicum* DSM 1731, a Solvent-Producing Strain with Multireplicon Genome Architecture, *J. Bacteriol.*, 2011, **193**(18), 5007–5008.
 - 30 S. Hu, *et al.*, Comparative genomic and transcriptomic analysis revealed genetic characteristics related to solvent formation and xylose utilization in *Clostridium acetobutylicum* EA 2018, *BMC Genomics*, 2011, **12**(1), 93.
 - 31 G. Gottschalk, *Regulation of Bacterial Metabolism*, Springer-Verlag, 1986.
 - 32 E. R. Kashket and J. S. Terracciano, Intracellular Conditions Required for Initiation of Solvent Production by *Clostridium acetobutylicum*, *Appl. Environ. Microbiol.*, 1986, **52**(1), 86–91.
 - 33 F. Monot, J. M. Engasser and H. Petit De Mange, Influence of pH and undissociated butyric acid on the production of acetone and butanol in batch cultures of *Clostridium acetobutylicum*, *Appl. Microbiol. Biotechnol.*, 1984, **19**(6), 422–426.
 - 34 J. B. Russell, Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation *versus* uncoupling, *J. Appl. Microbiol.*, 2008, **73**(5), 363–370.
 - 35 A. P. Mariano, *et al.*, Assessment of *in situ* butanol recovery by vacuum during acetone butanol ethanol (ABE) fermentation, *J. Chem. Technol. Biotechnol.*, 2012, **87**(3), 334–340.
 - 36 J. Liu, *et al.*, Enhanced butanol production by increasing NADH and ATP levels in *Clostridium beijerinckii* NCIMB 8052 by insertional inactivation of *Cbei_4110*, *Appl. Microbiol. Biotechnol.*, 2016, **100**(11), 4985–4996.
 - 37 Y. Gu, *et al.*, Utilization of economical substrate-derived carbohydrates by solventogenic *Clostridia*: pathway dissection, regulation and engineering, *Curr. Opin. Biotechnol.*, 2014, **29**, 124–131.
 - 38 S. B. Li, *et al.*, Pathway dissection, regulation, engineering and application: lessons learned from biobutanol production by solventogenic *Clostridia*, *Biotechnol. Biofuels*, 2020, **13**(1), 39.
 - 39 A. Sawisit, *et al.*, Optimization of sodium hydroxide pretreatment and enzyme loading for efficient hydrolysis of rice straw to improve succinate production by metabolically engineered *Escherichia coli* KJ122 under simultaneous saccharification and fermentation, *Bioresour. Technol.*, 2018, **260**, 348–356.
 - 40 S. H. Han, *et al.*, Biobutanol production from 2-year-old willow biomass by acid hydrolysis and acetone-butanol-ethanol fermentation, *Energy*, 2013, **61**, 13–17.
 - 41 S. Shanmugam, *et al.*, Enhanced bioconversion of hemicellulosic biomass by microbial consortium for biobutanol production with bioaugmentation strategy, *Bioresour. Technol.*, 2019, **279**, 149–155.
 - 42 S. Fatma, *et al.*, Lignocellulosic Biomass: A Sustainable Bioenergy Source for the Future, *Protein Pept. Lett.*, 2018, **25**(2), 148–163.
 - 43 K. Karimi, M. Shafei and R. Kumar, *Progress on physical and chemical pretreatment of lignocellulosic biomass*, Springer, Berlin, Heidelberg, 2013, pp. 53–96.



- 44 C. Birgen, *et al.*, Butanol production from lignocellulosic biomass: revisiting fermentation performance indicators with exploratory data analysis, *Biotechnol. Biofuels*, 2019, **12**, 167.
- 45 Y. R. Wu, *et al.*, Synergistic enzymatic saccharification and fermentation of agar for biohydrogen production, *Bioresour. Technol.*, 2017, **241**, 369–373.
- 46 M. Herbaut, *et al.*, Multimodal analysis of pretreated biomass species highlights generic markers of lignocellulose recalcitrance, *Biotechnol. Biofuels*, 2018, **11**, 52.
- 47 S. Sombatpraiwan, *et al.*, Optimization of microwave-assisted alkali pretreatment of cassava rhizome for enhanced enzymatic hydrolysis glucose yield, *Food and Energy Security*, 2019, **8**(4), e00174.
- 48 C. L. Cheng, *et al.*, Biobutanol production from agricultural waste by an acclimated mixed bacterial microflora, *Appl. Energy*, 2012, **100**, 3–9.
- 49 A. K. Kumar and S. Sharma, Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review, *Bioresour. Bioprocess.*, 2017, **4**, 7.
- 50 X. Kong, *et al.*, Biobutanol production from sugarcane bagasse hydrolysate generated with the assistance of gamma-valerolactone, *Process Biochem.*, 2016, **51**(10), 1538–1543.
- 51 H. Li, *et al.*, Enhanced enzymatic hydrolysis and acetone-butanol-ethanol fermentation of sugarcane bagasse by combined diluted acid with oxidate ammonolysis pretreatment, *Bioresour. Technol.*, 2017, **228**, 257–263.
- 52 C. Tang, *et al.*, Sustainable biobutanol production using alkali-catalyzed organosolv pretreated cornstalks, *Ind. Crops Prod.*, 2017, **95**, 383–392.
- 53 H. Su, *et al.*, A biorefining process: Sequential, combinational lignocellulose pretreatment procedure for improving biobutanol production from sugarcane bagasse, *Bioresour. Technol.*, 2015, **187**, 149–160.
- 54 K. Liu, *et al.*, Butanol production from hydrothermolysis-pretreated switchgrass: Quantification of inhibitors and detoxification of hydrolyzate, *Bioresour. Technol.*, 2015, **189**, 292–301.
- 55 S. K. Bhatia, *et al.*, Current status and strategies for second generation biofuel production using microbial systems, *Energy Convers. Manage.*, 2017, **148**, 1142–1156.
- 56 H. Shukor, *et al.*, Enhanced mannan-derived fermentable sugars of palm kernel cake by mannanase-catalyzed hydrolysis for production of biobutanol, *Bioresour. Technol.*, 2016, **218**, 257–264.
- 57 T. Bao, *et al.*, n-Butanol and ethanol production from cellulose by *Clostridium cellulovorans* overexpressing heterologous aldehyde/alcohol dehydrogenases., *Bioresour. Technol.*, 2019, **285**, 121316.
- 58 Y. T. Tan, A. S. M. Chua and G. C. Ngoh, Deep eutectic solvent for lignocellulosic biomass fractionation and the subsequent conversion to bio-based products - A review, *Bioresour. Technol.*, 2020, **297**, DOI: [10.1016/j.biortech.2019.122522](https://doi.org/10.1016/j.biortech.2019.122522).
- 59 H. Xu, *et al.*, Key process parameters for deep eutectic solvents pretreatment of lignocellulosic biomass materials: A review, *Bioresour. Technol.*, 2020, **310**, 123416.
- 60 X. Q. Lin, *et al.*, High-efficient cellulosic butanol production from deep eutectic solvent pretreated corn stover without detoxification, *Ind. Crops Prod.*, 2021, **162**, 113258.
- 61 A. Procentese, *et al.*, Pre-treatment and enzymatic hydrolysis of lettuce residues as feedstock for bio-butanol production, *Biomass Bioenergy*, 2017, **96**, 172–179.
- 62 K. R. Corbin, *et al.*, Grape marc as a source of carbohydrates for bioethanol: Chemical composition, pre-treatment and saccharification, *Bioresour. Technol.*, 2015, **193**, 76–83.
- 63 A. Karungi, A. Pogrebnoi and T. Kivevele, Optimization of microwave-assisted alkali pretreatment followed by acid hydrolysis of sugarcane straw for production of acetone-butanol-ethanol, *Energy Sources, Part A*, 2020, DOI: [10.1080/15567036.2020.1760404](https://doi.org/10.1080/15567036.2020.1760404).
- 64 M. O. Jang and G. Choi, Techno-economic analysis of butanol production from lignocellulosic biomass by concentrated acid pretreatment and hydrolysis plus continuous fermentation, *Biochem. Eng. J.*, 2018, **134**, 30–43.
- 65 B. Branska, *et al.*, Chicken feather and wheat straw hydrolysate for direct utilization in biobutanol production, *Renewable energy*, 2020, **145**, 1941–1948.
- 66 B. Zghari, L. Hajji and B. Abdellatif, Effect of Moist and Dry Heat Weathering Conditions on Cellulose Degradation of Historical Manuscripts exposed to Accelerated Ageing: ¹³C NMR and FTIR Spectroscopy as a non-Invasive Monitoring Approach, *J. Mater. Environ. Sci.*, 2018, **9**(2), 641–654.
- 67 W. Wang, *et al.*, Study on the decreased sugar yield in enzymatic hydrolysis of cellulosic substrate at high solid loading, *Appl. Biochem. Biotechnol.*, 2011, **164**(7), 1139–1149.
- 68 H. Amiri and K. Karimi, Pretreatment and hydrolysis of lignocellulosic wastes for butanol production: Challenges and perspectives, *Bioresour. Technol.*, 2018, **270**, 702–721.
- 69 M. Mirfakhar, *et al.*, Co-fermentation of hemicellulosic hydrolysates and starch from sweet sorghum by *Clostridium acetobutylicum*: A synergistic effect for butanol production, *Ind. Crops Prod.*, 2020, **151**, 112459.
- 70 S. A. Survase, *et al.*, Continuous acetone-butanol-ethanol fermentation using SO₂-ethanol-water spent liquor from spruce, *Bioresour. Technol.*, 2011, **102**(23), 10996–11002.
- 71 E. Wyman Charles, Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals (Wyman/Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals), *Laboratory Pretreatment Systems to Understand*, 2013, pp. 489–521, DOI: [10.1002/9780470975831](https://doi.org/10.1002/9780470975831).
- 72 N. Qureshi, *et al.*, Production of butanol (a biofuel) from agricultural residues: Part II - Use of corn stover and switchgrass hydrolysates, *Biomass Bioenergy*, 2010, **34**(4), 566–571.



- 73 A. Ranjan and V. S. Moholkar, Comparative study of various pretreatment techniques for rice straw saccharification for the production of alcoholic biofuels, *Fuel*, 2013, **112**, 567–571.
- 74 Z. J. Sun and S. J. Liu, Production of n-butanol from concentrated sugar maple hemicellulosic hydrolysate by *Clostridia acetobutylicum* ATCC824, *Biomass Bioenergy*, 2012, **39**, 39–47.
- 75 M. Theiri, *et al.*, Development of sequential and simultaneous bacterial cultures to hydrolyse and detoxify wood pre-hydrolysate for enhanced acetone-butanol-ethanol (ABE) production, *Enzyme Microb. Technol.*, 2020, **133**, 109438.
- 76 J. X. Zhang, *et al.*, Recent developments in ionic liquid pretreatment of lignocellulosic biomass for enhanced bioconversion, *Sustainable Energy Fuels*, 2021, **5**(6), 1655–1667.
- 77 Y. Zhang, *et al.*, The importance of engineering physiological functionality into microbes, *Trends Biotechnol.*, 2009, **27**(12), 664–672.
- 78 M. T. Mohedano, O. Konzock and Y. Chen, Strategies to increase tolerance and robustness of industrial microorganisms, *Synth. Syst. Biotechnol.*, 2022, **7**(1), 533–540.
- 79 M. S. Pino, *et al.*, Bioreactor design for enzymatic hydrolysis of biomass under the biorefinery concept, *Chem. Eng. J.*, 2018, **347**, 119–136.
- 80 I. S. Maddox, *et al.*, Production of acetone-butanol-ethanol from concentrated substrate using *clostridium acetobutylicum* in an integrated fermentation-product removal process - ScienceDirect, *Process Biochem.*, 1995, **30**(3), 209–215.
- 81 P. Narueworanon, *et al.*, Impacts of initial sugar, nitrogen and calcium carbonate on butanol fermentation from sugarcane molasses by *Clostridium beijerinckii*, *Energies*, 2020, **13**(3), 1–19.
- 82 H. Amiri and K. Karimi, Integration of autohydrolysis and organosolv delignification for efficient acetone, butanol, and ethanol production and lignin recovery, *Ind. Eng. Chem. Res.*, 2016, **55**(17), 4836–4845.
- 83 M. Yang, *et al.*, Enhanced acetone-butanol-ethanol production from lignocellulosic hydrolysates by using starchy slurry as supplement, *Bioresour. Technol.*, 2017, **243**, 126–134.
- 84 C. Ren, *et al.*, Identification and inactivation of pleiotropic regulator CcpA to eliminate glucose repression of xylose utilization in *Clostridium acetobutylicum*, *Metab. Eng.*, 2010, **12**(5), 446–454.
- 85 C. Lu, *et al.*, Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping, *Bioresour. Technol.*, 2012, **104**, 380–387.
- 86 H. Z. Luo, *et al.*, Recent advances and strategies in process and strain engineering for the production of butyric acid by microbial fermentation, *Bioresour. Technol.*, 2018, **253**, 343–354.
- 87 H. H. Liu, *et al.*, Omics-based analyses revealed metabolic responses of *Clostridium acetobutylicum* to lignocellulose-derived inhibitors furfural, formic acid and phenol stress for butanol fermentation, *Biotechnol. Biofuels*, 2019, **12**, 101.
- 88 E. Palmqvist and B. Hahn-Hagerdal, Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition, *Bioresour. Technol.*, 2000, **74**(1), 25–33.
- 89 D. H. Cho, S. J. Shin and Y. H. Kim, Effects of acetic and formic acid on ABE production by *Clostridium acetobutylicum* and *Clostridium beijerinckii*, *Biotechnol. Bioprocess Eng.*, 2012, **17**(2), 270–275.
- 90 S. H. Wang, *et al.*, Formic Acid Triggers the "Acid Crash" of Acetone-Butanol-Ethanol Fermentation by *Clostridium acetobutylicum*, *Appl. Environ. Microbiol.*, 2011, **77**(5), 1674–1680.
- 91 T. Ezeji, N. Qureshi and H. P. Blaschek, Butanol production from agricultural residues: Impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation, *Biotechnol. Bioeng.*, 2007, **97**(6), 1460–1469.
- 92 N. Qureshi, *et al.*, Effect of cellulosic sugar degradation products (furfural and hydroxymethyl furfural) on acetone-butanol-ethanol (ABE) fermentation using *Clostridium beijerinckii* P260, *Food Bioprod. Process.*, 2012, **90**(3), 533–540.
- 93 Z. Yan, H. Bei and T. C. Ezeji, Biotransformation of furfural and 5-hydroxymethyl furfural (HMF) by *Clostridium acetobutylicum* ATCC 824 during butanol fermentation, *New Biotechnol.*, 2012, **29**(3), 345–351.
- 94 S. Maiti, A re-look at the biochemical strategies to enhance butanol production, *Biomass Bioenergy*, 2016, **94**, 187–200.
- 95 W. H. Chen and Y. R. Zeng, Mathematical model to appraise the inhibitory effect of phenolic compounds derived from lignin for biobutanol production, *Bioresour. Technol.*, 2018, **261**, 44–51.
- 96 H. Q. Gu, *et al.*, Physiological mechanism of improved tolerance of *Saccharomyces cerevisiae* to lignin-derived phenolic acids in lignocellulosic ethanol fermentation by short-term adaptation, *Biotechnol. Biofuels*, 2019, **12**(1), 268.
- 97 T. Zhao, Y. Tashiro and K. Sonomoto, Smart fermentation engineering for butanol production: designed biomass and consolidated bioprocessing systems, *Appl. Microbiol. Biotechnol.*, 2019, **103**(23–24), 9359–9371.
- 98 H. Z. Luo, *et al.*, Co-production of solvents and organic acids in butanol fermentation by *Clostridium acetobutylicum* in the presence of lignin-derived phenolics, *RSC Adv.*, 2019, **9**(12), 6919–6927.
- 99 C. F. D. Vieira, *et al.*, Isopropanol-butanol-ethanol (IBE) production in repeated-batch cultivation of *Clostridium beijerinckii* DSM 6423 immobilized on sugarcane bagasse, *Fuel*, 2020, **263**, 116708.
- 100 S. Lee, J. H. Lee and R. J. Mitchell, Analysis of *Clostridium beijerinckii* NCIMB 8052's transcriptional response to ferulic acid and its application to enhance the strain tolerance, *Biotechnol. Biofuels*, 2015, **8**, 68.



- 101 M. P. Raut, *et al.*, Quantitative proteomic analysis of the influence of lignin on biofuel production by *Clostridium acetobutylicum* ATCC 824, *Biotechnol. Biofuels*, 2016, **9**, 113.
- 102 S. A. Survase, *et al.*, Efficient strategy to alleviate the inhibitory effect of lignin-derived compounds for enhanced butanol production, *ACS Sustainable Chem. Eng.*, 2021, **9**(3), 1172–1179.
- 103 L. J. Jönsson, Bioconversion of lignocellulose: inhibitors and detoxification, *Biotechnol. Biofuels*, 2013, **6**, 16.
- 104 L. Encina, *et al.*, Hydrogels derived from galactoglucomannan hemicellulose with inorganic contaminant removal properties, *RSC Adv.*, 2021, **11**(57), 35960–35972.
- 105 Z. Xiao, *et al.*, Effects of sugar inhibition on cellulases and β -glucosidase during enzymatic hydrolysis of softwood substrates, *Appl. Biochem. Biotechnol.*, 2004, **116**(1–3), 1115–1126.
- 106 M. Galbe and G. Zacchi, Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production, *Adv. Biochem. Eng./Biotechnol.*, 2007, **108**, 41–65.
- 107 V. B. Barua, V. V. Goud and A. S. Kalamdhad, Microbial pretreatment of water hyacinth for enhanced hydrolysis followed by biogas production, *Renewable Energy*, 2018, **126**, 21–29.
- 108 S. Maiti, *et al.*, Hydrolytic pre-treatment methods for enhanced biobutanol production from agro-industrial wastes, *Bioresour. Technol.*, 2018, **249**, 673–683.
- 109 Y. J. Jiang, *et al.*, Consolidated bioprocessing of butanol production from xylan by a thermophilic and butanogenic *Thermoanaerobacterium* sp. M5, *Biotechnol. Biofuels*, 2018, **11**, 89.
- 110 N. Qureshi, *et al.*, Removal of fermentation inhibitors from alkaline peroxide pretreated and enzymatically hydrolyzed wheat straw: Production of butanol from hydrolysate using *Clostridium beijerinckii* in batch reactors, *Biomass Bioenergy*, 2008, **32**(12), 1353–1358.
- 111 A. Boonsombuti, *et al.*, Enhancement of ABE fermentation through regulation of ammonium acetate and D-xylose uptake from acid-pretreated corncobs, *Ann. Microbiol.*, 2014, **64**, 431–439.
- 112 L. Yu, *et al.*, Metabolic engineering of *Clostridium tyrobutyricum* for n-butanol production through co-utilization of glucose and xylose, *Biotechnol. Bioeng.*, 2015, **112**(10), 2134–2141.
- 113 V. H. G. Diaz and G. O. Tost, Economic optimization of *in situ* extraction of inhibitors in acetone-ethanol-butanol (ABE) fermentation from lignocellulose, *Process Biochem.*, 2018, **70**, 1–8.
- 114 L. D. Gottumukkala, *et al.*, Biobutanol production from rice straw by a non acetone producing *Clostridium sporogenes* BE01, *Bioresour. Technol.*, 2013, **145**, 182–187.
- 115 L. J. Jonsson and C. Martin, Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects, *Bioresour. Technol.*, 2016, **199**, 103–112.
- 116 S. S. Hassan, G. A. Williams and A. K. Jaiswal, Emerging technologies for the pretreatment of lignocellulosic biomass, *Bioresour. Technol.*, 2018, **262**, 310–318.
- 117 N. Qureshi, *et al.*, Cellulosic butanol (ABE) biofuel production from sweet sorghum bagasse (SSB): impact of hot water pretreatment and solid loadings on fermentation employing *Clostridium beijerinckii* P260, *BioEnergy Res.*, 2016, **9**(4), 1167–1179.
- 118 L. Zhang, *et al.*, Ribulokinase and transcriptional regulation of arabinose metabolism in *Clostridium acetobutylicum*, *J. Bacteriol.*, 2012, **194**(5), 1055–1064.
- 119 J. Lee, *et al.*, Evidence for the presence of an alternative glucose transport system in *Clostridium beijerinckii* NCIMB 8052 and the solvent-hyperproducing mutant BA101, *Appl. Environ. Microbiol.*, 2005, **71**(6), 3384–3387.
- 120 C. Voigt, H. Bahl and R. J. Fischer, Identification of PTS(Fru) as the major fructose uptake system of *Clostridium acetobutylicum*, *Appl. Microbiol. Biotechnol.*, 2014, **98**(16), 7161–7172.
- 121 W. Mitchell and M. Tangney, Carbohydrate uptake by the phosphotransferase system and other mechanisms, *Handbook on Clostridia*, 2005.
- 122 Z. Wen, *et al.*, Recent advances in metabolic engineering of clostridia for n-butanol production, *Synth. Biol. J.*, 2021, **2**(2), 194–221.
- 123 A. Hamid and K. Keikhosro, Pretreatment and Hydrolysis of Lignocellulosic Wastes for Butanol Production: Challenges and Perspectives, *Bioresour. Technol.*, 2018, **270**, S0960852418312215.
- 124 M. Yang, *et al.*, Co-fermentation of hemicellulose and starch from barley straw and grain for efficient pentoses utilization in acetone-butanol-ethanol production, *Bioresour. Technol.*, 2015, **179**, 128–135.
- 125 Y. Chen, *et al.*, Production of butanol from glucose and xylose with immobilized cells of *Clostridium acetobutylicum*, *Biotechnol. Bioprocess Eng.*, 2013, **18**(2), 234–241.
- 126 Y. D. Wu, *et al.*, Synergistic effect of calcium and zinc on glucose/xylose utilization and butanol tolerance of *Clostridium acetobutylicum*, *FEMS Microbiol. Lett.*, 2016, **363**(5), fnw023.
- 127 T. Zhao, *et al.*, Semi-hydrolysis with low enzyme loading leads to highly effective butanol fermentation, *Bioresour. Technol.*, 2018, **264**, 335–342.
- 128 Y. D. Wu, *et al.*, Impact of zinc supplementation on the improved fructose/xylose utilization and butanol production during acetone-butanol-ethanol fermentation, *J. Biosci. Bioeng.*, 2016, **121**(1), 66–72.
- 129 X. Zhao, *et al.*, A quantitative metabolomics study of high sodium response in *Clostridium acetobutylicum* ATCC 824 acetone-butanol-ethanol (ABE) fermentation, *Sci. Rep.*, 2016, **6**, 28307.
- 130 Y. Zhang, Y. Zhu, Y. Zhu and Y. Li, The importance of engineering physiological functionality into microbes, *Trends Biotechnol.*, 2009, **27**(12), 664–672.
- 131 G. de Gonzalo, *et al.*, Bacterial enzymes involved in lignin degradation, *J. Biotechnol.*, 2016, **236**, 110–119.



- 132 C. H. Zhao, *et al.*, Design and development of a “Y-shaped” microbial consortium capable of simultaneously utilizing biomass sugars for efficient production of butanol, *Metab. Eng.*, 2019, **55**, 111–119.
- 133 C. L. Tri and I. Kamei, Butanol production from cellulosic material by anaerobic co-culture of white-rot fungus *Phlebia* and bacterium *Clostridium* in consolidated bioprocessing, *Bioresour. Technol.*, 2020, **305**, 123065.
- 134 D. Haldar and M. K. Purkait, Lignocellulosic conversion into value-added products: A review, *Process Biochem.*, 2020, **89**, 110–133.
- 135 V. Parisutham, T. H. Kim and S. K. Lee, Feasibilities of consolidated bioprocessing microbes: From pretreatment to biofuel production, *Bioresour. Technol.*, 2014, **161**, 431–440.
- 136 C. R. He, Y. Y. Kuo and S. Y. Li, Lignocellulosic butanol production from Napier grass using semi-simultaneous saccharification fermentation, *Bioresour. Technol.*, 2017, **231**, 101–108.
- 137 T. G. Li, Y. R. Wu and J. Z. He, Heterologous expression, characterization and application of a new beta-xylosidase identified in solventogenic *Clostridium* sp. strain BOH3, *Process Biochem.*, 2018, **67**, 99–104.
- 138 Y. D. Wu, *et al.*, High temperature simultaneous saccharification and fermentation of corn stover for efficient butanol production by a thermotolerant *Clostridium acetobutylicum*, *Process Biochem.*, 2021, **100**, 20–25.
- 139 N. Qureshi, B. C. Saha and M. A. Cotta, Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part II - Fed-batch fermentation, *Biomass Bioenergy*, 2008, **32**(2), 176–183.
- 140 M. Seifollahi and H. Amiri, Enhanced production of cellulosic butanol by simultaneous co-saccharification and fermentation of water-soluble cellulose oligomers obtained by chemical hydrolysis, *Fuel*, 2019, **263**(2823), 116759.
- 141 M. Balat, Production of bioethanol from lignocellulosic materials *via* the biochemical pathway: A review, *Energy Convers. Manage.*, 2011, **52**(2), 858–875.
- 142 A. Valles, *et al.*, Fed-batch simultaneous saccharification and fermentation including in-situ recovery for enhanced butanol production from rice straw, *Bioresour. Technol.*, 2021, **342**, 126020.
- 143 D. G. Olson, *et al.*, Recent progress in consolidated bioprocessing, *Curr. Opin. Biotechnol.*, 2012, **23**(3), 396–405.
- 144 Y. J. Jiang, *et al.*, Recent advances of biofuels and biochemicals production from sustainable resources using co-cultivation systems, *Biotechnol. Biofuels*, 2019, **12**, 155.
- 145 P. P. Lin, *et al.*, Consolidated bioprocessing of cellulose to isobutanol using *Clostridium thermocellum*, *Metab. Eng.*, 2015, **31**, 44–52.
- 146 T. Bao, *et al.*, n-Butanol and ethanol production from cellulose by *Clostridium cellulovorans* overexpressing heterologous aldehyde/alcohol dehydrogenases, *Bioresour. Technol.*, 2019, **285**, 121316.
- 147 L. Tian, *et al.*, Metabolic engineering of *Clostridium thermocellum* for n-butanol production from cellulose, *Biotechnol. Biofuels*, 2019, **12**, 186.
- 148 F. Salimi and R. Mahadevan, Characterizing metabolic interactions in a clostridial co-culture for consolidated bioprocessing, *BMC Biotechnol.*, 2013, **13**, 95.
- 149 S. Nakayama, *et al.*, Butanol production from crystalline cellulose by cocultured *Clostridium thermocellum* and *Clostridium saccharoperbutylacetonicum* N1-4, *Appl. Environ. Microbiol.*, 2011, **77**(18), 6470–6475.
- 150 Y. J. Jiang, *et al.*, Consolidated bioprocessing performance of a two-species microbial consortium for butanol production from lignocellulosic biomass, *Biotechnol. Bioeng.*, 2020, **117**(10), 2985–2995.
- 151 J. Zheng, *et al.*, Recent advances to improve fermentative butanol production: Genetic engineering and fermentation technology, *J. Biosci. Bioeng.*, 2015, **119**(1), 1–9.
- 152 Z. Chang, *et al.*, Effective multiple stages continuous acetone-butanol-ethanol fermentation by immobilized bioreactors: Making full use of fresh corn stalk, *Bioresour. Technol.*, 2016, **205**, 82–89.
- 153 Y. Ni, *et al.*, Continuous butanol fermentation from inexpensive sugar-based feedstocks by *Clostridium saccharobutylicum* DSM 13864, *Bioresour. Technol.*, 2013, **129**, 680–685.
- 154 X. Chi, *et al.*, Features of a staged acidogenic/solventogenic fermentation process to improve butanol production from rice straw, *Energy Fuels*, 2019, **33**(2), 1123–1132.
- 155 D. Sahoo, *et al.*, Effect of dilute acid pretreatment of wild rice grass (*Zizania latifolia*) from Loktak Lake for enzymatic hydrolysis, *Bioresour. Technol.*, 2018, **253**, 252–255.
- 156 L. J. Jonsson, B. Alriksson and N. O. Nilvebrant, Bioconversion of lignocellulose: inhibitors and detoxification, *Biotechnol. Biofuels*, 2013, **6**, 16.
- 157 J. Singh, M. Suhag and A. Dhaka, Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: A review, *Carbohydr. Polym.*, 2015, **117**, 624–631.
- 158 T. Zhao, *et al.*, Semi-hydrolysate of paper pulp without pretreatment enables a consolidated fermentation system with *in situ* product recovery for the production of butanol, *Bioresour. Technol.*, 2019, **278**, 57–65.
- 159 C. Xue, *et al.*, The advanced strategy for enhancing biobutanol production and high-efficient product recovery with reduced wastewater generation, *Biotechnol. Biofuels*, 2017, **10**, 148.
- 160 Y. Wu, *et al.*, Pleiotropic regulation of a glucose-specific PTS in *Clostridium acetobutylicum* for high-efficient butanol production from corn stover without detoxification, *Biotechnol. Biofuels*, 2019, **12**, 264.
- 161 Z. Q. Wen, *et al.*, Metabolic Engineering of *Clostridium cellulovorans* to Improve Butanol Production by



- Consolidated Bioprocessing, *ACS Synth. Biol.*, 2020, **9**(2), 304–314.
- 162 A. Ranjan, S. Khanna and V. S. Moholkar, Feasibility of rice straw as alternate substrate for biobutanol production, *Appl. Energy*, 2013, **103**, 32–38.
- 163 K. Gao and L. Rehmann, ABE fermentation from enzymatic hydrolysate of NaOH-pretreated corncobs, *Biomass Bioenergy*, 2014, **66**, 110–115.
- 164 F. Xia, *et al.*, An integrated biorefinery process to produce butanol and pulp from corn straw, *Ind. Crops Prod.*, 2019, **140**, 111648.
- 165 K. Gao, *et al.*, Cellulosic Butanol Production from Alkali-Pretreated Switchgrass (*Panicumvirgatum*) and Phragmites (*Phragmites australis*), *Bioresour. Technol.*, 2014, **174**, 176–181.
- 166 X. Sun, *et al.*, Feasibility of using biochar as buffer and mineral nutrients replacement for acetone-butanol-ethanol production from non-detoxified switchgrass hydrolysate, *Bioresour. Technol.*, 2020, **298**, 122569.
- 167 I. Valdez-Vazquez, *et al.*, Hydrogen and butanol production from native wheat straw by synthetic microbial consortia integrated by species of *Enterococcus* and *Clostridium*, *Fuel*, 2015, **159**, 214–222.
- 168 G. X. Qi, *et al.*, Enhanced butanol production from ammonium sulfite pretreated wheat straw by separate hydrolysis and fermentation and simultaneous saccharification and fermentation, *Sustain. Energy Technol. Assess.*, 2019, **36**, 100549.
- 169 N. Qureshi, M. A. Cotta and B. C. Saha, Bioconversion of barley straw and corn stover to butanol (a biofuel) in integrated fermentation and simultaneous product recovery bioreactors, *Food Bioprod. Process.*, 2014, **92**(C3), 298–308.
- 170 S. V. Avula, S. C. Reddy and L. V. Reddy, The Feasibility of Mango (*Mangifera indica* L.) Peel as an Alternative Substrate for Butanol Production, *Bioresources*, 2015, **10**(3), 4453–4459.
- 171 F. Raganati, *et al.*, Butanol Production by Fermentation of Fruit Residues, *Chem. Eng. Trans.*, 2016, **49**, 229–234.
- 172 D. Johnravindar, *et al.*, Waste-to-biofuel: production of biobutanol from sago waste residues, *Environ. Technol.*, 2017, **38**(13–16), 1725–1734.
- 173 N. Thieme, *et al.*, Milling byproducts are an economically viable substrate for butanol production using clostridial ABE fermentation, *Appl. Microbiol. Biotechnol.*, 2020, **104**(20), 8679–8689.
- 174 Q. He and H. Z. Chen, Increased efficiency of butanol production from spent sulfite liquor by removal of fermentation inhibitors, *J. Cleaner Prod.*, 2020, **263**, 121356.
- 175 M. L. Xia, *et al.*, Development of optimal steam explosion pretreatment and highly effective cell factory for bioconversion of grain vinegar residue to butanol, *Biotechnol. Biofuels*, 2020, **13**(1), 111.
- 176 Q. Jin, *et al.*, High acetone-butanol-ethanol production from food waste by recombinant *Clostridium saccharoperbutylacetonicum* in batch and continuous immobilized-cell fermentation, *ACS Sustainable Chem. Eng.*, 2020, **8**(26), 9822–9832.
- 177 N. Qureshi, *et al.*, Production of acetone-butanol-ethanol (ABE) from concentrated yellow top press cake using *Clostridium beijerinckii* P260, *J. Chem. Technol. Biotechnol.*, 2020, **95**(3), 614–620.
- 178 L. V. Reddy, A. S. Veda and Y. J. Wee, Utilization of banana crop residue as an agricultural bioresource for the production of acetone-butanol-ethanol by *Clostridium beijerinckii* YVU1, *Lett. Appl. Microbiol.*, 2020, **70**(1), 36–41.
- 179 A. M. Zetty-Arenas, *et al.*, Towards enhanced n-butanol production from sugarcane bagasse hemicellulosic hydrolysate: Strain screening, and the effects of sugar concentration and butanol tolerance, *Biomass Bioenergy*, 2019, **126**, 190–198.
- 180 W. Tao, *et al.*, Introducing transglutaminase with its precursor region into *Clostridium acetobutylicum* improves its tolerance to oxidative stress and solvent production, *Process Biochem.*, 2015, **50**(1), 111–118.
- 181 B. Bharathiraja, *et al.*, Biobutanol - An impending biofuel for future: A review on upstream and downstream processing techniques, *Renewable Sustainable Energy Rev.*, 2017, **68**, 788–807.
- 182 A. Sanchez, *et al.*, Bidimensional sustainability analysis of lignocellulosic ethanol production processes. Method and case study, *Biofuels, Bioprod. Biorefin.*, 2014, **8**(5), 670–685.
- 183 I. Valdez-Vazquez, *et al.*, A comparison of biological, enzymatic, chemical and hydrothermal pretreatments for producing biomethane from Agave bagasse, *Ind. Crops Prod.*, 2020, **145**, 112160.
- 184 S. H. Shen, *et al.*, Enhanced alcohol titre and ratio in carbon monoxide-rich off-gas fermentation of *Clostridium carboxidivorans* through combination of trace metals optimization with variable-temperature cultivation, *Bioresour. Technol.*, 2017, **239**, 236–243.
- 185 S. Chakraborty, *et al.*, Enrichment of a solventogenic anaerobic sludge converting carbon monoxide and syngas into acids and alcohols, *Bioresour. Technol.*, 2019, **272**, 130–136.
- 186 V. Muller, New horizons in acetogenic conversion of one-carbon substrates and biological hydrogen storage, *Trends Biotechnol.*, 2019, **37**(12), 1344–1354.

