

Biobutanol Production from Lignocellulosic Biomass: Prospective and Challenges

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Abstract

Biobutanol production from lignocellulosic biomass is considered promising and economically feasible. This paper provides an updated review on development of lignocellulose-based biobutanol production with a focus on the understanding of the structure of the feedstock, pretreatment technologies, and fermentation processes. To enhance butanol production from lignocellulosic materials, strategies in terms of inhibitors detoxification, strains improvement and process integration and optimization are also addressed. Besides, the reviewer attempts to shed light on the challenges and perspectives for the bioconversion of lignocellulosic biomass-to-butanol.

Keywords: Butanol; Lignocellulose; Pretreatment; Fermentation; Bioconversion

Introduction

Considering the fast depletion of fossil fuel-reserves and environmental impact caused by the over-consumption of fossil-based energy, there is an urgent need to develop renewable and environmental friendly alternatives to substitute fossil fuels. Butanol is an idea alternative due to its significant properties such as high energy content, low volatility and hygroscopicity [1]. In particular, it can be used directly or blended with gasoline without the need to change any current vehicles and supplied with existing gasoline pipes [2]. Of the butanol production ways, biological butanol (biobutanol) production by microbial fermentation has been widely concerned [3,4]. Nevertheless, the major obstacle to the commercialization of such a sustainable pathway is the high production cost [4,5]. Currently, biobutanol is mainly produced from conventional sugar-and starch-based feedstocks such as sugarcane, molasses and corn [6]. Taking into account the global population and increased demand for food supply, this feedstock is not sustainable and cost-effective. Therefore, the development of alternative feedstocks that are widely available at low cost and abundant supply is the key issue.

Lignocellulosic biomass, such as agricultural and forest residues, is by far the most abundant and sustainable renewable natural resource on the earth. Particularly, as one of the biggest agricultural countries in the world, China has abundant lignocellulosic biomass resources. It's reported that nearly 0.73 billion tons of agricultural residues are produced per year, the value is equivalent to 12,000 trillion kJ of energy. Moreover, there is approximately 37 million m³ of forest wastes, which contained 580 trillion kJ of energy [7-9]. Unfortunately, up to now, most of the biomass residuals are burned directly. This not only has low energy efficiency, but also causes serious environmental pollution such as respiratory aerosols, SO₂ and CO. Therefore, the conversion of lignocellulosic biomass to high quality bioenergy by advanced processes rather than combustion is a promising way to substitute fossil-based fuels and decrease greenhouse gas emissions [10,11]. However, there are challenges in producing butanol from lignocellulosic biomass due to their recalcitrance to degradation [12] as well as their unique chemical compositions. It has been acknowledged that the use of lignocellulosic biomass involves pretreatment and hydrolysis of raw material followed by fermentation of sugars to butanol. The process can be made cost-effective by choosing an efficient pretreatment, hydrolysis method, and

fermentation strategy. Although several studies have demonstrated the potential bioconversion of lignocellulosic biomass to butanol after pretreatment, there is still lack of systematic study on bioconversion lignocellulosic biomass to butanol to the authors' best knowledge.

In this paper, an up-to-date review on the bioconversion of lignocellulosic biomass to butanol in terms of the characteristics of the feedstock, pretreatment technologies, fermentation processes and strategies for enhancing butanol production. Remaining challenges and perspectives for the conversion of lignocellulosic biomass to butanol are also presented.

Structure of Lignocellulosic Biomass

Lignocellulosic biomass is a complex substrate. Understanding of its characteristics, particularly its chemical compositions, is a prerequisite for developing efficient pretreatment technologies to deconstruct its rigid structure and conditioning subsequent enzymatic hydrolysis of cellulose to liberate sugars, as well as designing microorganisms to convert sugars into butanol with high yields.

Lignocellulosic material is mainly composed of cellulose, hemicellulose and lignin, which are associated with each other [13]. However, the composition of these constitutes varies widely with plant species, growth of stage and area, time of harvest and other conditions, e.g., softwoods have higher content of cellulose, whereas grass and leaves have more hemicellulose (Table 1).

Cellulose

Cellulose is the most abundant and interesting polysaccharide in biomass [15]. Its molecule is composed of D-glucose subunits that are linked by β -1,4-glycosidic bonds with cellulobiose as the repeating unit. Fermentable D-glucose can be produced from cellulose by breaking the β -1, 4-glycosidic linkages.

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Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Paper	85-99	0	0-15
Wheat straw	30	50	15
Rice straw	32.1	24	18
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seeds hairs	80-95	May-20	0
Newspaper	40-55	25-40	18-30
Waste paper from chemical pulps	60-70	Oct-20	05-Oct
Fresh bagasse	33.4	30	18.9
Swine waste	6	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Switch grass	45	31.4	12

NA, data not available; ^aAdapted from Ref. [14]

Table 1: Chemical compositions of various lignocellulosic biomass^a.

The cellulose in biomass consists of two forms: one is organized structure called crystalline cellulose and the other is not well-organized structure called amorphous cellulose [16]. Crystalline structure comprises the major proportion of cellulose, whereas small amount of unorganized structure forms amorphous cellulose. Compared to crystalline cellulose, amorphous cellulose is more susceptible to enzyme. Appropriate measures are therefore needed to change crystalline structure cellulose in biomass into amorphous polymorphs that can be hydrolyzed more efficiently by cellulases. **Hemicellulose**

Different from cellulose, hemicellulose is a more complex carbohydrate structure that consists of different polymers including pentoses (such as xylose and arabinose), hexoses (such as glucose and galactose), and uronic acids (such as 4-omethylglucuronic and D-galactouronic acids) [17]. In biomass, hemicelluloses are embedded and interact with cellulose and serve as a connection between the lignin and the cellulose fibers which make the whole cellulose-hemicellulose-lignin network more strength and toughness. In contrast to the resistance of crystalline cellulose, the polymers present in hemicellulose are more sensitive to thermal-chemically pretreatment and easy to be hydrolyzed to monomer sugars even though they cocrystallize with cellulose chains [18].

Lignin

Of the three components, lignin which presents in the cellular wall is the most recalcitrant to degradation due to its highly organized structure. It is an amorphous heteropolymer consisting of three major monolignols (p-coumaryl, coniferyl and sinapyl alcohols) that are linked together by alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds. Lignin is not a sugar-based polymer and cannot be employed as substrate for biobutanol production. However, it exhibits a significant influence on the economic and efficient performance of the bioconversion processes, since most undesirable inhibitors of microbial growth and fermentation released during the pretreatment from this compound [19].

Pretreatment of Lignocellulosic Materials

It is acknowledged that the presence of lignin and hemicellulose makes the cellulose more difficult to cellulases attack. For improving the conversion of lignocellulosic biomass to biobutanol, pretreatment is required to alter the structure and chemical composition of the

biomass, so that the hydrolysis efficiency of the carbohydrate to fermentable sugars can be enhanced with high yield and rate [20,21]. In the past decades, various pretreatments methods, roughly divided into four categories: physical pretreatment, chemical pretreatment, solvent fractionation and biological decomposition, have been developed to improve hydrolysis rates [22]. As an ideal pretreatment process, it should maximize sugars yield from pretreated biomass, and in the meantime minimize energy consumption and environmental impact. However, none of them can meet all these criteria. For example, current widely used chemical pretreatment methods like acid/alkaline hydrolysis and ozonolysis which require excessive use of chemicals such as concentrated acids [22,23], alkaline and oxidizing agents that may interfere in the subsequent hydrolysis [24]. Moreover, these chemicals are toxic, corrosive and hazardous, which make the pretreatment more costly due to the need of anti-corrosion equipment. Similarly, physical-chemical methods like steam explosion, ammonia fiber expansion (AFEX), and wet air oxidation which are operated at high temperature and pressure, making the process energy-intensive and economic unfeasible [25]. In addition, operations at such high temperature and pressure lead to the formation of toxic compounds such as furfural and 5-hydroxymethylfurfural (HMF) derived from sugar degradation, acetate from hemicellulose, and a number of soluble aromatic compounds (acids, aldehydes, and alcohols) from lignin, which are known to be inhibitory to enzymatic hydrolysis and fermentation [26,27]. Thus the major challenge for pretreatment is to avoid the degradation or loss of carbohydrate, and minimize the cost of treatment so as to make the process economically feasible.

Table 2 lists the advantages and disadvantages of various pretreatments [28]. Of all pretreatments, a combination of different

Pretreatment process	Advantages	Limitations and disadvantages
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	Not efficient for biomass with high lignin content
CO ₂ explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Biological	Degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low

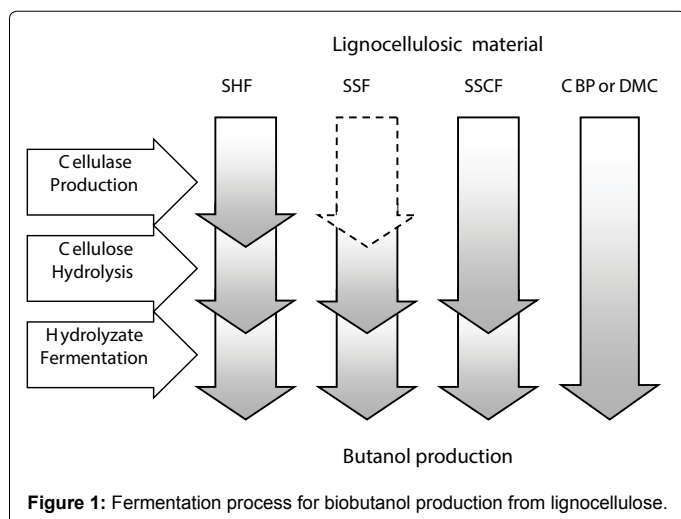
Table 2: Comparative evaluation of different pretreatment strategies.

pretreatments by taking advantage of their specific superiorities might be favorable since the limitations of one pretreatment maybe overcome by the other pretreatment used in the combination. For instance, sulfur-catalyzed steam explosion in which an external acid was added to aid solubilize hemicellulose, this can help lower the optimal pretreatment temperature and pressure and give a better enzymatic hydrolysis ratio [29,30]. In another combination of biological pretreatment using white-rot fungi to treat rice straw followed with AFEX pretreatment gave significantly higher glucan and xylan conversions and less-severe AFEX conditions than with AFEX only [31]. In addition, compared with physical and chemical pretreatments in which excess chemicals and water and intensive energy consumption are needed, biological pretreatment by employing microorganisms to degrade lignocellulosic biomass at mild condition with the advantages of high specificity and low energy requirement maybe another cost-effective strategy [32].

As mentioned above, lignocellulosic biomass is a complex feedstock whose physicochemical properties and chemical compositions vary with different biomass. It should be noted that it is not always possible to apply the conditions of pretreatment from one type of material to another. In other words, one pretreatment method is efficient for a particular type of material might not work for another material [33]. It therefore can be concluded that the choice of appropriate pretreatment technology and its optimization for a particular biomass depends on the physicochemical properties and chemical compositions of biomass as well as which components of the biomass need to be altered. Additionally, although a variety of studies on optimization of pretreatment at bench-scale have been carried, not much research on optimization of pretreatment at scale-up has been done. Since the results obtained at bench-scale may not feasibly meet the situation at pilot-scale. Thus, it is suggested that much effort should be paid on selection and optimization of pretreatment strategy at scale up.

Fermentation Processes

Following pretreatment, enzymatic hydrolysis is needed to convert cellulose into glucose, which can be subsequently used for butanol fermentation [34]. Based on the production of cellulase and the process of cellulose hydrolysis and butanol fermentation, the conversion of lignocellulosic biomass for butanol production can be classified into four major processes: (1) separate hydrolysis and fermentation (SHF), (2) simultaneous saccharification and fermentation (SSF), (3) simultaneous saccharification and co-fermentation (SSCF), and (4) consolidated bioprocessing (CBP) as illustrated schematically in Figure 1.



Separate hydrolysis and fermentation

For the separate hydrolysis and fermentation (SHF) process, cellulose is hydrolyzed to glucose by cellulases under optimum conditions, particularly around 50°C, and then resulting glucose is transferred to fermentation reactor and fermented by butanol-producing microorganisms at temperatures around 35°C [35]. As summarized in Table 3, the process of SHF is currently the most frequently applied in butanol production from lignocellulosic biomass. For example, Cheng et al. evaluated the butanol production from rice straw and sugarcane bagasse by a mixture culture using SHF [10]. Wang et al. discussed the feasibility of using crude enzyme to hydrolyze wheat straw for butanol fermentation in SHF processes [5]. In SHF process, cellulose hydrolysis and fermentation can be carried out at their different optimum conditions. However, the accumulation of glucose during enzymatic hydrolysis may inhibit cellulases activity in the hydrolysis process. Moreover, two separated enzymatic hydrolysis and fermentation processes inevitably increase the cost of equipment and the risk of microbial contamination which makes the process uneconomically feasible.

Simultaneous saccharification and fermentation

Different from SHF, simultaneous saccharification and fermentation (SSF) is the process that cellulose hydrolysis and fermentation takes place in one step. When a similar process is applied in butanol production from lignocellulosic biomass taking into account the unique properties of the hydrolysate that includes both C5 and C6 sugars, the term simultaneous saccharification and co-fermentation (SSCF) is used [36].

Compared with SHF, SSF process has the advantages of lower equipment cost and sugar inhibition. However, the temperatures for enzymatic hydrolysis and butanol fermentation are significantly different, because the optimum temperature for cellulose hydrolysis by cellulases normally occurs at 50°C, while the optimum temperature for butanol-producing strain is around 35°C. This makes the simultaneous optimization of the two unit operations impossible [37]. Generally, the SSF process for butanol production is operated at lower temperatures to accommodate microbial growth and butanol fermentation, usually at 35-37°C. Consequently, the efficiency of the enzymatic hydrolysis is inevitably compromised, and a much longer time is needed to complete the hydrolysis.

Consolidated bioprocessing

Although the processes of SHF and SSF are currently the most widely employed strategies to produce butanol from lignocellulosic biomass, one of the major barriers for the SHF and SSF is the exogenous cellulase addition which increases the cost of butanol production due to the high cost of the cellulase as well as the high enzyme dosage required by the processes [38]. Thus, development of cost-effective and efficient saccharification and fermentation technologies is the key for successful cellulosic butanol production.

An alternative process that aims to eliminate this critical cost-increasing is the consolidated bioprocessing (CBP) also called direct microbial conversion, in which cellulase production, cellulose hydrolysis and fermentation are completed in one step [39,40]. This integrated process has been proposed as the most economically attractive configuration for low cost hydrolysis and fermentation of cellulosic biomass. It is estimated that CBP has a potential for cost reduction in excess of 50% compared to the processes of SSF and SHF due to the elimination of the operating and capital costs correlated with

Microorganism	Fermentation process	Substrate	Butanol concentration (g/L)	Butanol yield (g/g substrate)	Butanol productivity (g/L·h)	References
Acclaimed mixed bacterial microflora	SHF	Rice straw	2.92 g/L,	-	0.042	[32]
		Sugarcane bagasse	2.29 g/L	-	0.059	
<i>C. thermocellum</i> + <i>C. saccharoperbutylacetonicum</i> N1-4	CBP	Crystalline cellulose	7.9	0.198	0.037	[33]
<i>C. beijerinckii</i> BA101	SHF	Corn fiber	6.4	0.138	0.073	[34]
<i>C. beijerinckii</i> NCIMB8052	SHF	Corn cob	5.6	0.13	0.057	[35]
<i>C. saccharoperbutylacetonicum</i> N1-4	SHF	Sago starch	10.4	0.29	0.072	[36]
<i>C. acetobutylicum</i> MTCC 481	SHF	Rice straw	2.1	1.04	0.017	[37]
<i>C. acetobutylicum</i> ATCC824	SSF	Seepweed	3.5	0.101	0.101	[38]
<i>C. beijerinckii</i> P260	SSF	Wheat straw	7.4	0.113	0.164	[39]
	SHF		8.09	0.193	0.085	
<i>C. acetobutylicum</i> ATCC824	SHF	Wheat straw	7.05	0.155	0.141	[5]
	SSF		5.05	0.127	0.08	

SHF: Separate hydrolysis and fermentation; SSF: Simultaneous saccharification and fermentation; CBP: Consolidated bioprocessing

Table 3: Biobutanol production from cellulosic substrates.

the additional steps of cellulase production and enzymatic hydrolysis [40,41]. However, there is no perfect CBP microorganism that can degrade lignocellulosic biomass efficiently and at the same time produce butanol at desired yield. Co-cultures have been widely studied to address the limitations in substrate utilization by individual strains for the production of various bioproducts. For example, it was reported that the co-culture of *Bacillus* sp. SGP1 and *C. tyrobutyricum* ATCC 25755T to produce butyric acid from sucrose [42]. Geng et al. discussed the effect of key factors on hydrogen production from cellulose in a co-culture of cellulolytic bacterium *Clostridium thermocellum* DSM 1237 and a non-cellulolytic hydrogen-producing bacterium *Clostridium thermopalmarium* DSM 5974 [43]. Apparently, co-culture of different microorganisms by taking advantage of their specific metabolic capacities provides a promising method to improve the substrate conversion and the product yield. Recently, Dwidar et al. evaluated the efficiency of butanol production from crystalline cellulose via CBP by cocultured cellulolytic *Clostridium. thermocellum* JN4 and the butanol-producing strain *Clostridium. saccharoperbutylacetonicum* N1-4. After 9d fermentation, butanol yield as high as 7.9 g/L was obtained on 4% Avicel cellulose [32]. To the best of authors' knowledge, this is the first report to produce butanol from cellulosic materials via CBP, indicating that much effort should be paid on butanol production through CBP strategy. Overall, development of CBP microorganisms is the core of the CBP process.

Strategies for Increasing Butanol Production from Lignocellulose

Inhibitors detoxification

Besides monomeric sugars are produced during the pretreatment and hydrolysis steps, some undesirable degradation products, including aliphatic acids (acetic, formic and levulinic acids), furan derivatives (furfural, 5-hydroxymethyl-furfural (5-HMF)), and phenolic compounds (such as syringaldehyde, ferulic and p-coumaric acids) [44,45], are also generated. These inhibitors have been identified to exert a significant influence on the effectiveness of biomass conversion [46,47], removal of these inhibitory compounds before fermentation is therefore pivotal for successful biofuel production. Traditional methods for the removal of inhibitors mostly employ the processes of adsorption, extraction, precipitation and their combinations e.g., vacuum evaporation and overliming with calcium hydroxide [48,49]. Biologic abatement (or bio-abatement) can be an alternative method

to reduce inhibitory compounds from biomass hydrolysates [50-52]. Bioabatement, in which inhibitors are removed by microbial metabolism, has the advantage of treating liquid-solid mixtures without the need of chemical inputs and with no generation of chemical wastes. Cao et al. utilized a fungus, *Coniochaeta ligniaria* NRRL30616, to treat dilute acid and liquid hot water-pretreated corn stover liquors and found most acetate and more than 50% of HMF, furfural, and phenolic compounds were successfully removed [50].

Although the detoxification for the removal of inhibitors from lignocellulosic hydrolysates has been proven to improve their fermentability, only part of inhibitors were removed in most cases. So, the selection of appropriate detoxification method depends on the type of hydrolysate to be treated with respect to the efficiency of the detoxification on that kind of hydrolysate and the fermentation microorganisms employed in the butanol production with respect to which inhibitors need to be removed.

Strain improvement

It is acknowledged that one of the biggest challenges for industrialization of butanol fermentation is low butanol yield due to the formation of byproducts acetone and ethanol and low butanol tolerance capability of the microorganism. To overcome this drawback, developing robust strains with improved butanol yield and tolerance is the prerequisite for butanol fermentation. Aiming at this, a lot of works have been made on modifying strains through mutagenesis, evolutionary engineering and metabolic engineering strategies e.g., a mutant strain *C. beijerinckii* BA101 treated N-methyl-N-nitro-N-nitrosoguanidine could produce 19 g/L butanol, which was 10 g/L higher than by the parent strain *C. beijerinckii* NCIMB 8052 [53], a another hyper-butanol producing strain *C. acetobutylicum* JB200, acquired from *C. acetobutylicum* ATCC 55025 by long term adaptation with intermittently butanol challenges, could tolerate and produce butanol as high as about 20 g/L [54], and a recombinant strain disrupted of acetoacetate decarboxylase gene *adc* significantly increased the butanol ratio from 70% to 80% [55]. In addition, it should be noted that the hydrolysates of lignocellulosic biomass contain pentose sugars such as xylose and arabinose and hexose sugars of glucose, mannose and galactose. Unfortunately, current butanol-producing microorganisms either *C. acetobutylicum* or *C. beijerinckii* cannot ferment the pentose sugars into butanol efficiently. If only hexose sugars from lignocellulosic biomass are fermented with pentose sugars left behind, feedstock consumption for biobutanol production will be significantly

high. Therefore engineering pentose-utilizing microorganisms with butanol pathways or butanol producers with pentose-metabolizing pathways is preferred.

Despite the progress made at molecular and physio-ecological levels, a superior butanol producing phenotypes with wide substrates spectrum especially have a capability to utilize pentose and cellulose at high rate still remains to be expected.

Process integration and optimization

Like ethanol production from lignocellulose-based feedstock, butanol production from this material involves various technologies such as pretreatment, saccharification and fermentation. In order to achieve an economical butanol production system, process integration aiming at optimizing these units on the system level is of great important. Considerable successes on the development of ethanol system in the aspects of pretreatment, enzymatic hydrolysis, fermentation, and ethanol recovery have been achieved in the past few years [56]. These successful cases may be good references for the butanol production from lignocellulosic material. In addition, many novel ideas, such as biorefinery and the concept of oriented conversion of classified composition which have been proposed and investigated in ethanol production may also provide practical and promising strategies for developing a feasible butanol production technology from lignocellulosic biomass [12,56,57]. Whatever, to achieve the commercialization of cellulosic butanol, further decrease in the cost of individual process and efficient combination of these processes will result in competitive biobutanol production from biomass.

Conclusions

Bioconversion of lignocellulose-to-butanol provides a sustainable and economical way to produce butanol from raw material via biotechnology. While, a deep understanding of fundamentals of various pretreatment processes and development of more efficient and economical fermentation processes needs relentless effort. Moreover, the development of cost-effective detoxification, more efficient microbial strains and process integration and optimization for reducing energy consumption and increasing butanol yields from raw materials would decrease the cost of butanol production and make it more economically competitive.

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