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REVIEW PAPER

Recent advances in development of biomass pretreatment technologies used in biorefinery for the production of bio-based fuels, chemicals and polymers

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Abstract−Biochemical conversion of biomass into biofuels, biochemicals, and biopolymers has attracted much interest throughout the world in terms of biorefineries. Lignocellulosic biomass is one of the most plentifully available biomass resources on the earth. It is composed of three main biopolymers - cellulose, hemicelluloses, and lignin, all of which are cross-linked to each other to resist degradation by enzymes and microorganisms resulting in so-called biomass recalcitrance. The biorefinery process typically consists of three steps: pretreatment, hydrolysis, and fermentation. Energy and cost efficiency of biorefinery is predominantly dependent on how to produce inexpensive sugars from complex cell wall component of lignocellulosic biomass by overcoming biomass recalcitrance. There have been tremendous efforts to develop effective biomass pretreatment technologies for obtaining the highest yield of fermentable sugars from biomass feedstocks at the lowest cost. The present review discusses various pretreatment technologies to understand how to effectively break down biomass into fermentable sugars that are eventually used for microbial fermentation to produce biomass-based fuels, chemicals, and polymers.

Keywords: Biorefinery, Lignocellulosic Biomass, Pretreatment, Biofuels, Biochemicals

INTRODUCTION

Owing to the increasing environmental problem of the CO_2 emissions and demand for alternatives to fossil fuels, integrated utilization of biomass for biochemical conversion to produce biofuels, biochemical, and biopolymers has attracted worldwide attention [1]. Biomass is generally considered as materials that are biologically derived from living organisms, and recently refers to plant-derived materials such as trees, algae, and agricultural crops [2]. Biomass, a sustainable and renewable energy resource, is commonly considered as an alternative to fossil-based resources. Total global production of biomass reaches up to 170 billion tons per year, but of which only small portions are currently being harvested, cultivated, and used as food and non-food [3]. Among a variety of biomass resources, lignocellulosic biomass has received great intention, because it is one of the most plentifully available resources on the earth. It includes terrestrial plant, energy crops, and agricultural/forestry residue, and of which annual global production is estimated to 10-50 billion tons based on dry biomass [4]. It is composed of cellulose, hemicelluloses, and lignin along with minor components such as ash, proteins and extractives. The compositional amount of the three main components varies depending on the biomass species, but cellulose generally accounts for 40-60%, hemicelluloses for 20-40% and lignin for 15-25%, respectively, based on the dry biomass. In terms of biorefinery, the various components in lignocellulosic biomass can be converted to biofuels, biopolymers and platform biochemicals through biochemical conversion consisting of pretreatment, enzymatic hydrolysis, and microbial fermentation. The most common resources that have been examined for producing these products are plant-derived biomass. Production of biofuels such as ethanol, butanol, and isobutanol from sugar and starch-based biomass, and even from the lignocellulosic biomass has extensively been examined due to their relatively low cost, great abundance, and sustainable supply [5,6].

However, there are many bottlenecks to limit conversion of the lignocellulosic biomass to desired products due to the biomass recalcitrance. Biomass recalcitrance is attributed to its structure rigidity and complexity via the spatial interaction of its chemical compositions, such as cellulose, hemicelluloses, and lignin, which results in protecting the biomass against enzyme and microbial attack. Pretreatment is crucial for the development of biorefinery employing microorganisms as host strains for the production of bioproducts by fermentations since it can provide fermentable sugars by dis-

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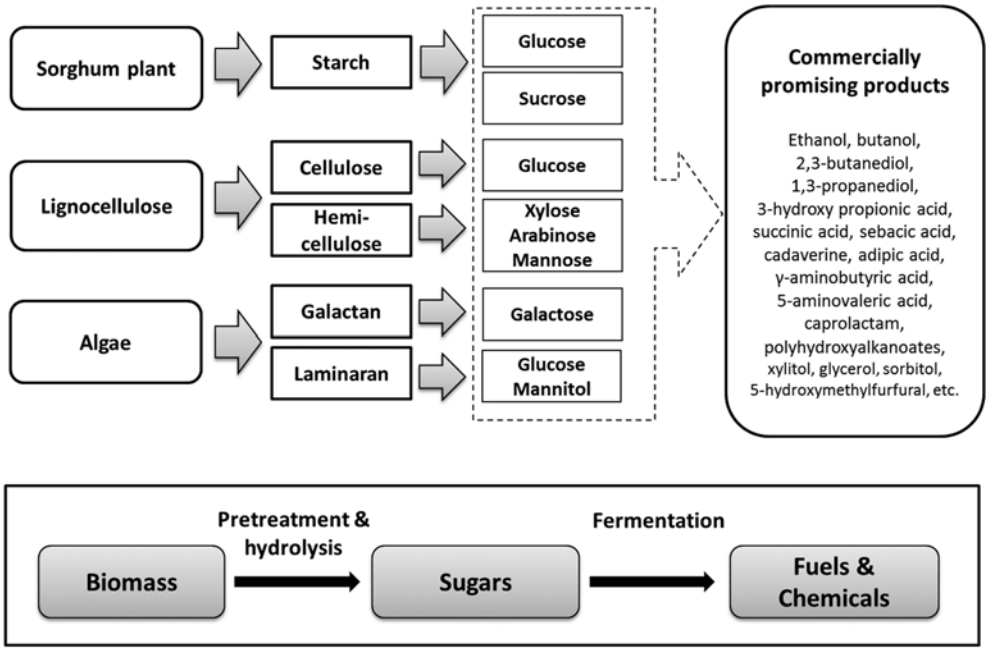

Fig. 1. An overview of the biomass-based biorefinery industry for the production of biofuels, bio-based platform chemicals and biopolymers from different biomass resources.

rupting the biomass recalcitrance. The main objective of the pretreatment is to break down plant cell wall in the biomass resulting in increasing susceptibility of cellulose to cellulolytic enzymes. Pretreatment technologies of biomass have been used to convert biomass into fermentable sugars through physical, chemical and biological processes such as alkali pretreatment and enzymatic hydrolysis. The ultimate goal of the pretreatment is to break down cell wall in the biomass for increasing susceptibility of cellulose to enzyme. It is important to achieve the highest yield of sugars with the lowest yield of inhibitory products at the lowest costs through effective pretreatment of the biomass having less reduced size [7].

Pretreatment strategies need to differ depending on biomass sources due to their differences in chemical compositions and structures. An overview of the biorefinery industry for producing biofuels, and biochemicals from different biomass sources is described in Fig. 1. In this review, various pretreatment technologies applied to a number of the lignocellulosic biomass to convert it into fermentable sugars will be discussed with their current results, which are eventually employed for the production of biofuels, biopolymers and biochemicals through microbial fermentation processes.

BIOMASS RESOURCES

1. Sorghum Plants

Sorghum plants such as sugar beet, sweet sorghum, and sugarcane have high sugar content. These plants are usually used for producing first generation bioethanol. After sugar extraction from the plant, sugar syrup can be directly fermented by microorganisms into biofuels such as ethanol as well as biochemicals. Some sorghum plants have sucrose as the main component such as sugarcane and sugar beets [8-10]. Other sugar source is starch, a polysaccharide consisting of glucose units joined by α-1,4- and α-1,6-glycosidic linkages (amylose and amylopectin) and is commonly found in wheat, maize, and barley [11]. These crops can be harvested 1-2 times per year, and their simple chemical structures make processing straightforward [12]. However, sorghum is one of the most important crops widely used for foods in Africa, Central America, and South Asi; thus, production of biofuels from food crops can cause conflicts in food and land use for the edible biomass and various social problems related to food shortages. Hence, non-food crops such as agricultural wastes and energy crops have received great attention in respect to the production of biofuels, biopolymers and biochemicals from the alternative resources to edible biomass.

2. Lignocellulosic Biomass

Non-food biomass such as woody and agricultural waste can be used for the production of second generation biofuels and biochemicals. It mostly consists of lignocellulosic biomass. Cellulose, hemicelluloses, and lignin are the three main components of lignocellulosic biomass including woody biomass, agricultural biomass, and herbaceous biomass [13]. Although compositional amounts of each vary depending on the lignocellulosic biomass species, cellulose, hemicelluloses, and lignin generally amount to about 35-50%, 15-35%, and 15-25%, respectively, on the dry weight basis. Lignocellulosic biomass also contains other minor non-structural components such as proteins, ash, and extractives [5,14].

Cellulose, a polysaccharide derived from β-1,4-glycosidic linked glucose unit, is the main cell wall component and the most abundant natural polymer on earth. It is in charge of supporting structural rigidity of the biomass through the formation of long and oriented microfibrils by intra- and intermolecular hydrogen bonding between parallel chains of polysaccharide. The cellulose microfibrils generate a high-ordered crystalline structure that is recalcitrant to enzyme and microbial attack [11]. Contrary to the starch, much low pH,

high reaction temperatures, or high residence times are required to hydrolyze significant portion of cellulose to glucose due to the crystalline structure of cellulose.

Hemicelluloses, branched polysaccharides that are derived from various pentose and hexose sugars, are main components along with cellulose in most of the lignocellulosic biomass, and their compositions and structures vary depending on the biomass sources. The representative polysaccharide of hemicelluloses in hardwoods is an O-acetyl-4-O-methylglucuronoxylans, of which content reaches up to 15-30% depending on the species. The typical hemicellulose isolated from softwoods, however, is an O-acetyl-galactoglucomannan with content of 10-25%, whereas that of cereal straw, a herbaceous biomass, consists of arabinoxylans [15]. Amorphous and branched hemicellulose with single chain structure is less recalcitrant to be much more susceptible to hydrolysis by acids than cellulose. Also, the extent of removal of hemicelluloses from cellulose matrix can affect cellulose accessibility to enzyme, since they are crosslinked with the cellulose matrix [5].

As one of the main structural polymers, lignin is a three-dimensional amorphous polyphenolic polymer that is derived from phenyl propane units (p-coumaryl, coniferyl, and sinapyl alcohol) via radical coupling polymerization catalyzed by oxidative enzyme. The relative portions of each monomer in lignin are different depending on biomass species. Lignin is mainly found in the secondary cell wall of plants. Lignin provides mechanical strength of the cell wall and chemical barrier to microbial attack and has significant role in conducting water in plant stems due to its hydrophobic structure [16]. Since lignin acts as the concrete that fills the remaining gap and holds the polysaccharides in the cell wall, it is considered as major recalcitrance for biomass conversion [5].

Each of the components within the cell walls such as cellulose and lignin can be recalcitrant to enzyme and microbial attack. The recalcitrance is particularly more enhanced due to the complexity in which cell wall components such as lignin, hemicelluloses and proteins are linked together resulting in a complex matrix. There are many problems in converting biomass to fermentable sugars that are to be used for the microbial fermentations for the production of biofuels and biochemicals due to the biomass recalcitrance. Therefore, recalcitrance should be reduced prior to biological conversion such as enzymatic hydrolysis of the biomass to efficiently isolate fermentable sugars as much as possible from lignocellulosic biomasses.

3. Algae

Algae have recently attracted extensive attention throughout the world and have been regarded as one of new biomass sources for producing bio-based products. They have distinguishing features from the lignocellulosic biomasses, such as non-competitiveness with land crops, high productivity (biomass/area/time), and a large proportion of starch or oil in algal population [17,18]. In addition, neither fresh water nor expensive supplements are necessary for algae production since they grow well on nutrients in sewage.

Algae are roughly classified into two categories, so called macro- and microalgae. Macroalgae are broadly composed of red-, green-, and brown macroalgae [19]. Since the carbohydrate content in several species is as high as that in land plants, it is reasonable to expect that there are many possibilities in converting the carbohydrates into valuable chemicals with high yields and productivities [12,20-24]. However, at present, biogas is the only product that deserves notice obtained from macroalgae [25]. On the other hand, relatively large numbers of species comprise microalgae with diverse constituents. Since microalgae are small and exist as single cells, massive cultivation of microalgae is easier and more controllable than that of macroalgae. However, subsequent harvesting is more difficult due to their small size [26]. Both groups are not covered in depth in this paper, but it can be easily accessible to numerous informative sources since a number of studies have been focused entirely on converting into biofuels and useful products from the biomass. Compositions of typical raw materials belonging to the three biomass types are summarized in Table 1.

BIOMASS PRETREATMENT

One of the essential processing steps for the production of fermentable sugars from lignocellulosic biomass is pretreatment that

Table 1. Chemical composition of different biomass types

Biomass type		Composition (% dry weight)				References
		Starch	Cellulose	Hemicellulose	Lignin	
Starch	Corn	72	0	0	0	[109]
Lignocellulosic	Switchgrass	0	31	22	23	[110]
	Bagasse	3	38	27	20	[2]
	Hardwood stems	0	40-55	24-40	18-25	[28]
	Softwood stems	0	45-50	25-35	25-35	[28]
	Corn cobs	0	45	35	15	[28]
	Wheat straw	0	30	50	15	[28]
Algae	*G. amansii*	0	17	59[a]	nr.	[12]
	L. japonica	0		52[b]	nr.	[102]
	S. fulvellum	0		40[b]	nr.	[102]

*nr., Not reported

[a]In terms of galactan

[b]Total carbohydrates

breaks down the cell wall structures resulting in enhancing accessibility of cellulose to enzyme during enzymatic hydrolysis. The main goal for biomass pretreatment is to enhance accessibility of cellulose to cellulases, resulting in maximizing enzymatic digestibility of cellulose. It has been generally recognized that biomass pretreatment is the most expensive processing step demanding high operating and processing costs among the entire biorefinery processes. Therefore, it is important to comprehend how to effectively open up the biomass structure to overcome the recalcitrance during biomass pretreatment at the lowest costs, and to develop optimizing conditions of the pretreatment for less generation of inhibitory products that interfere with downstream operations such as enzymatic hydrolysis and microbial fermentation.

To date, a number of pretreatment technologies have been investigated to achieve the highest fermentable sugars yields from the lignocellulosic biomass. Due to the diversity of pretreatment methods, it is important to selectively operate the methods that are effective in achieving the highest yield of products for downstream operations at the lowest costs. The pretreatment performance is to be evaluated by considering following factors: (a) high susceptibility of cellulose to cellulolytic enzymes; (b) high recovery of hemicellulosic sugars; (c) low capital and operating costs; (d) low energy input; (e) low yield of biological inhibitors; and (f) low cost of chemicals [27].

According to the pretreatment mechanisms for breaking down biomass recalcitrance, in general, the various pretreatment methods can be categorized into four main categories (a) physical, (b) biological, and (c) chemical, and (d) physico-chemical pretreatment [28]. To enhance pretreatment performance at lower pretreatment severity, recently, combined pretreatment has been tried. In the following sections, various pretreatment technologies are discussed, and it is also described how chemical and physical effects disrupting biomass recalcitrance are different depending on the pretreatments.

1. Physical Pretreatment

Physical pretreatment such comminution, extrusion, and irradiation mechanically breaks down the ultrastructure of biomass for improved enzymatic hydrolysis of cellulose. Physical pretreatment can reduce the particle size and crystallinity of biomass, leading to increase of the surface area and decrease of polymerization degree of lignocellulose [29,30]. Comminution pretreatment, e.g., chipping, grinding, and milling by using pulverizing apparatus such as hammer mills, knife mills, disc refiners, and planers, reduces the final particle size of lignocellulosic biomass up to 0.2-2 mm, and facilitates handling and treatment of feedstock [31]. However, decreasing the final particle size is energy intensive (~33% of the total electricity used in the whole process [32]) and thus requires other physical or chemical pretreatments, making mechanical comminution economically infeasible. Extrusion pretreatment is performed by multiple operations such as heating, mixing, and shearing in the extruder, resulting in physical and chemical modifications of lignocellulosic biomass [33]. According to biomass characteristics, extrusion pretreatment can be easily optimized by process modifications such as adding chemicals or organic solvents and changing screw speed or barrel temperature. In addition to comminution and extrusion, physical pretreatment includes thermal pretreatment with freeze/thaw, pyrolysis, and cryomilling or irradiation pretreatment with gamma ray, microwave, electron beam, and laser [30, 34]. Current irradiation methods are expensive and have many technical difficulties in industrial application such as operational unreliability or inability of scale up. However, a microwave method has been extensively examined as an alternative to conventional heating in combination with acid or alkaline pretreatment due to its low energy requirement by a large heating volume, rapid heating, and short treatment time [35].

Most of the physical pretreatments hardly reduce the biomass recalcitrance but require high operating and/or capital costs. Alternately, certain physical pretreatments can be used for post-treatment increasing enzymatic digestibility of pretreated biomass with combination of chemical pretreatments, which will be more discussed below. Also, physical treatment as biomass size reducing process is a prerequisite for preparing materials prior to conducting other biological or chemical pretreatments.

2. Biological Pretreatment

In contrast to physical and chemical pretreatments, biological pretreatment is conducted under mild conditions without chemicals, high temperature or pressure. Biological pretreatment by using enzymes or microorganisms has the following advantages over physical and chemical treatments [36]: it can efficiently degrade lignin, is environment-friendly and requires low energy input, and produces fewer inhibitors for enzymatic hydrolysis or microbial fermentation such as 2-furaldehyde (FF), 5-hydroxymethyl-2-furfural (HMF), or organic acids. In microbial pretreatment, lignin-degrading fungi such as white-, brown-, soft-rot fungi can preferentially degrade lignin from complex cell wall components and increase the efficiency of enzymatic hydrolysis of lignocellulose. In particular, the white-rot fungus *Phanerochaete chrysosporium* has been extensively investigated for microbial pretreatment due to its high growth rate and degradation rate of lignin [37]. In addition to fungi, some bacteria such as actinomycetes also exhibit a weak lignin-degrading capability [38]. The lignin-degrading microorganisms utilize oxidative enzymes such as laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase for selective degradation of lignin and accessory enzymes such as glyoxal oxidase and aryl alcohol oxidase for the production of hydrogen peroxide, an oxidant for lignin oxidation [39,40]. However, these lignin-degrading enzymes can be only exploited *in vitro* degradation of lignin model compounds due to their low activity and stability and narrow substrate specificity against lignin compounds.

Despite certain advantages of biological pretreatment, there are still many challenges in its industrial application. For example, the white-rot fungi can efficiently degrade lignin but simultaneously consume cellulose and hemicelluloses for their growth resulting in significant loss of fermentable sugars. Moreover, biological pretreatment requires longer pretreatment time and larger space than physical and chemical pretreatment resulting in high risk of contamination during the lignin degradation and increase of the capital cost. To overcome these limitations, it is necessary to develop designer microorganisms for implementing new lignolytic enzymes, removing cellulase and hemicellulase enzymes, and altering central metabolism for simple nutrition requirement by using bioinformatic tools, metagenomics tools, and high throughput screening.

3. Chemical Pretreatments

In chemical pretreatment, various chemicals can be used as catalyst for disrupting biomass recalcitrance to increase cellulose accessibility to cellulases [41]. Chemical pretreatment technologies using some acids, alkalis, and organic solvents have been investigated for a long time; consequently, some have been well developed at an industrial scale. These methods generally require high temperature ranging of 140-210 °C except for the alkaline pretreatment; thus they are also called thermochemical pretreatment. The chemical pretreatments as currently promising technologies can be broadly categorized into following methods: (1) alkaline pretreatment; (2) acid pretreatment; (3) sulfite pretreatment; (4) organosolv pretreatment; and (5) ionic liquid pretreatment. Their optimized pretreatment conditions differ from which chemicals are used for biomass pretreatment, so it is important to design various factors such as reactor type, chemical concentrations, reaction temperatures and time. Moreover, it is important to understand how these pretreatments make lignocellulosic biomass to be more suitable for enzymatic hydrolysis, because chemical and physical actions during the pretreating biomass vary depending on applied chemicals. In the following section, some promising chemical pretreatment technologies are summarized.

3-1. Alkaline Pretreatment

As for alkaline pretreatment, alkaline compounds such as potassium hydroxide (KOH), sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) also known as lime, and aqueous ammonia are used as catalysts for opening up biomass structure, especially by solubilizing a large portion of lignin in the alkali solutions. The alkaline pretreatment is usually carried out at relatively lower temperatures (85-150 °C) compared to the acid pretreatment, but longer pretreatment times of 3-13 h should be required for effectively disrupting the lignocellulosic biomass. Recently, the effects of three alkali catalysts, NaOH, KOH and $Ca(OH)_2$, on pretreatment of sweet sorghum stalks have been examined based on their lignin removal and sugar yields [42]. The results showed that NaOH and KOH could remove more than 70% of the lignin in sweet sorghum stalks by rather high alkali loading (more than 3.33 mmol/g dry biomass), whereas $Ca(OH)_2$ only removed lignin up to 44% when it was loaded by 2.5 mmol/g dry biomass [42]. The extent of xylan removal was increased up to 35% proportional to alkali loading of NaOH and KOH, but less than 15% of xylan was removed by loading high amount of $Ca(OH)_2$ up to 6.67 mmol/g dry biomass [42]. The study suggested 2.5 mmol/g dry biomass of NaOH as optimized alkali pretreatment condition, in which carbohydrate recovery reached as high as 92% of the sweet sorghum stalks with removal of 62% of lignin, and enzymatic digestibility of the glucan was 92% at enzyme loading of 10 FPU/g glucan [42].

Pretreatment method combined with steam explosion and alkaline pretreatment, in which steam explosion is firstly applied and then alkaline pretreatment is subsequently employed, has also been tried to effectively increase enzymatic digestibility of soft wood through removal of the large portion of hemicelluloses and lignin. The enzymatic digestibility of cellulose in the resulting biomass was increased by 30% compared to that of the biomass treated by only steam explosion [43]. Additionally, lime pretreatment using $Ca(OH)_2$ has been applied to the lignocellulosic biomass, which showed similar pretreatment performance with those using NaOH and KOH, but lime pretreatment has some advantages in terms of chemical cost, hazard, and recovery [44].

3-2. Acid Pretreatment

Acid pretreatment has received much attention as a promising process to be developed on an industrial scale. The main effect of the acid pretreatment on the reducing biomass recalcitrance is thata significant portion of hemicelluloses can be removed from biomass, which makes cellulose more accessible to cellulases. Either concentrated or dilute acid can be used in acid pretreatment, but the latter is more used due to its advantages such as less equipment corrosion and higher acid recovery, which positively affects reduction of operating and maintenance costs [45]. Contrary to alkali pretreatments, the dilute acid pretreatment is usually conducted at relatively higher temperatures of 160-220 °C, but requires shorter reaction time within several minutes. The acid pretreatment with highly severe conditions tends to cause some drawbacks such as the generation of further degraded products to be acted as inhibitors to render cellulolytic enzymes and fermentative microorganisms inactive at even lower temperatures. As main inhibitors from further degradation of biomass, there are FF from pentoses, HMF from hexoses, and organic acids from the biomass [46]. For example, the first model of hydrolysis of cellulose and degradation of glucose in dilute acid at high temperature has been proposed on the following series of first-order reactions basis [46].

$$\text{Cellulose} \xrightarrow{k_1} \text{glucose} \xrightarrow{k_2} \text{HMF} \tag{1}$$

where

$$k_i = H_i C_a^M \exp\left(\frac{-\Delta H_{a,i}}{RT}\right) \tag{2}$$

where k_i is the reaction rate constant (min^{-1}) for reaction i, H_i is a constant, C_a is the concentration of H_2SO_4 (%) and M is the reaction order, $\Delta H_{a,i}$ is the activation energy, R is the universal gas constant, and T is the absolute temperature. This model equation has generally been applied to describe hydrolysis kinetics of cellulose and hemicellulose under acid conditions.

Among the various acid pretreatments, dilute sulfuric acid (H_2SO_4) pretreatment has been investigated and developed as the most common method for converting the lignocellulosic biomass to fermentable sugars. This pretreatment can effectively reduce the biomass recalcitrance through the structural alterations such as hemicellulose removal, change in cellulose crystallinity, and increasing biomass porosity [47]. Lloyd and Wyman [48] focused on degradation behaviors of hemicellulose during acid pretreatment, and found that its large removal extent can open up more plant cell walls and make cellulose more accessible to cellulases, which can also be found in other studies [49,50]. Moreover, xylan and xylooligomers have been suggested to be strong inhibitors to cellulases, because they decreased cellulase reactivity to cellulose, resulting in the decrease of enzyme digestibility [51,52]. The anatomical structures of plant cell wall have an influence on cellulase accessibility to cellulose. The cellulases can access the cellulose surface through the pores present in the plant cell wall. A pore size larger than 3 nm makes cellulases more accessible to cellulose due to similar size of *T. reesei* cellulose complex with the pores [53]. Hence, it has been found to increase

specific area, pore volume and pore size distributed in pretreated biomass, which could be attributed from lignin re-distribution as well as large removal of hemicelluloses during dilute acid pretreatment [54,55]. The removal extent of lignin under acidic conditions can be changed depending on reactor configurations. The dilute acid pretreatment carried out in a batch-type reactor can slightly remove less than 15% of lignin from biomass, whereas the dilute acid pretreatment in flow through reactors pumping hot aqueous sulfuric acid through a column packed with biomass can substantially remove lignin up to 50% [56,57]. As electron microscopic techniques have been further developed, structural change in lignin during the dilute acid pretreatment can be described by images. Due to its hydrophobicity, lignin tends to amalgamate with each other into droplet-like structures rather than be soluble into water at high temperatures ranging from 90 to 190 °C, at which lignin can be melted [58]. After cooling, these lignin droplets adsorb on the surface of cellulose's microfibrils and remain as the droplets within cell wall matrix. The lignin migration affects the pore size and pore volume capable of diffusing cellulases into or out of the cell wall matrix. From these results, it is important for disrupting the lignocellulosic biomass to optimize conditions of dilute acid pretreatment making cellulose more accessible to enzyme through the structural and compositional modifications that can be varied depending on process conditions such as reaction temperatures, times, and acid concentrations.

3-3. Sulfite Pretreatment

Sulfite pretreatment, one of sulfite pulping processes, has been well optimized to pretreat the lignocellulosic biomass, especially soft wood such as pines. This sulfite pretreatment is referred to as sulfite pretreatment to overcome recalcitrance of lignocelluloses, which is abbreviated to SPORL process. Sulfite pretreatment has an economical and technological benefit, because it can be carried out using the existing pulping equipment and be applied to well-developed infrastructures in the pulp and paper industry for a long time [59]. In the SPORL process, wood chip is loaded to high-pressurized reactor and then soaked in acidic solution prepared by mixtures of bisulfite and sulfuric acid. This process is usually conducted at 180 °C for 30 min, after which pretreated chip is fibrillated by mechanical disk refining [59]. During the SPORL process, large portion of hemicelluoses is solubilized in liquid stream and some lignin fractions is sulfonated, which makes the SPORL process more attractive since enzyme dosage and water consumption can be reduced. It was found that when pretreated slurry without solid-liquid separation was subjected to enzymatic hydrolysis, its enzymatic digestibility was gradually increased with elevating pH from 4.5 to 5.5, which was comparable to that of washed pretreated biomass. This result implied that with increasing pH for enzymatic hydrolysis, electrostatic repulsion between cellulases and sulfonated lignin could be enhanced, so nonspecific binding of cellulases to lignin seemed to be reduced [60]. However, it should be more investigated whether it causes an inhibition of various microorganisms when the resulting fermentable source is directly used for microbial fermentation.

3-4. Organosolv Pretreatment

Similar to the SPORL process, organosolv pretreatment originated from organosolv pulping process that has an advantage of less effect on environmental pollution such as contaminating water and causing odor over kraft and sulfite pulping. It has been investigated which organic solvents can effectively break down the lignocellulosic biomass and how much enzymatic digestibility can be increased depending on organosolv pretreatment severity [61]. Among the various organic solvents used for biomass pretreatment such as methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol, etc., ethanol and methanol are commonly employed for pretreatment of the biomass due to their lower cost, easier recovery/recycle and higher miscibility with water. In the organosolv pretreatment, the lignocellulosic biomass is soaked in either organic solvents or aqueous solutions, and then heated to temperatures ranging of 100-250 °C for a variable period of time [28]. The organosolv pretreatment considerably changes physico-chemical properties of biomass: both lignin and hemicelluloses can be solubilized by cleavage of ester linkages between lignin-carbohydrates complex (LCC), which can be promoted at higher pretreatment severity [62]. Due to removal of hemicelluloses and lignin, a cellulose-rich solid can be obtained after solid-liquid separation followed by washing that is essential to remove soluble fractions enriched with lignin and hemicelluloses. Otherwise, these soluble fractions may cause an inhibition of enzymes and microorganisms [63]. In terms of biomass biorefinery, the organosolv pretreatment is one of the promising processes able to fractionate the lignocellulosic biomass into each component, which can be directly used as biochemicals and be further modified for use as an alternative to petroleum based chemicals. Particularly, various studies have focused on organosolv lignin recovered from liquid stream of organosolv treated biomass, because the lignin seems to have similar physico-chemical properties in nature, and can be used as renewable source for composite manufacturing [61,64]. However, organosolv pretreatment still has a number of barriers related to capital and operating costs, which limits its development at an industrial scale [28].

3-5. Ionic Liquid Pretreatment (IL Pretreatment)

Ionic liquids (ILs) are composed of organic cations and anions. Because ILs can be designed for use, they are recognized as "designer solvents" [65,66]. The ILs can dissolve cellulose at moderate temperatures and/or under ambient pressures, because the anions with high hydrogen-bond basicity can disrupt the cellulose crystalline. Additionally, the ILs can dissolve various biopolymers, such as lignin, starch, protein, and chitin/chitosan, and their dissolution extents are dependent on how to combine each of cation and anion of ILs [66]. In recent years, a number of studies have used the ILs for disrupting lignocellulosic biomass. It has been found that 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) and 1-ehtyl-3-methylimidazolium acetate ([Emim][CH_3COO]) can afford to effectively dissolve cellulose and lignocellulosic biomass at moderate temperatures ranging of 90-130 °C for a variable period of time (~24 h) and/or under pressures [67]. After which, the dissolved cellulose in liquid stream can be selectively recovered by precipitation with water. Prior to enzymatic hydrolysis, however, the precipitated cellulose should be thoroughly washed with water and/or acetone until no residual ILs remain. This regenerated cellulose become more amorphous; thus it is more accessible to cellulases.

On the other hand, Dordick and coworkers have shown that selective dissolution of lignin present in wood flour by 1-ethyl-3-meth-

yllimidazolium acetate ([Emim][CH_3COO]), which was carried out at 90 °C for variable a period of time ranging of 0-70 h could increase cellulose accessibility to cellulases.. In this strategy, cellulose-rich solid fraction could be obtained with less crystalline structure, whereas most of lignin was dissolved in a liquid phase and remained as unaltered native lignin. From such a treatment, more than 90% of cellulose can be hydrolyzed by cellulases [68].

In spite of high prices, the pretreatment using ILs has an advantage of reduced operating costs, because the ILs can be recovered up to more than 99% by either vacuum evaporation or formation of an aqueous biphasic system, and reused [69-71]. In addition, toxic compounds are less generated during the IL pretreatment, which helps to simplify waste disposal [72].

However, the IL's dissolution capacity of cellulose is considerably reduced in the presence of water, which could be attributed to competition of water and Cl^- anion of the IL to form hydrogen-bonding with the cellulose microfibrils. It was found that the IL was not able to dissolve cellulose in the presence of water amounting to 1 wt% in the IL. Therefore, it is difficult to effectively pretreat the lignocellulosic biomass by ILs containing Cl^- anions because of moisture in the biomass [73]. Moreover, some ILs containing Cl^- anions are also known to make cellulases become inactive [74]. To commercially use ILs in pretreating biomass, therefore, it should be designed for ILs to dissolve cellulose but also less inhibit cellulases and microorganisms. Then, it should be determined whether residual ILs in fermentable source have any toxic effects on enzymes and fermentative microorganisms depending on their amounts.

The main features of the chemical pretreatment technologies described above are summarized in Fig. 2.

4. Physico-chemical Pretreatment

4-1. Steam Explosion

Steam explosion is one of the promising physico-chemical pretreatment technologies. During steam explosion, the structure of lignocellulosic biomass is physically altered and its compositions are also chemically modified. The procedure of steam explosion is as follows. The biomass powder loaded in high pressurized reactor is heated to high temperatures (200-260 °C) by high-pressured saturated steam (2,000-5,000 kPa), then saturated for a few minutes. After that, the pressure valve is opened to reduce the pressure at which explosive expansion of moisture in the biomass occurs, and then biomass fibers are fibrilated [75]. A large extent of hemicelluloses can be auto-hydrolyzed during the stream explosion without adding any catalysts, because water itself acts as an acid at high temperatures and it is promoted due to liberated acetic acid from acetylated hemicelluloses moiety. Contrary to hemicelluloses, lignin is hardly removed during steam explosion, but its distribution on the biomass fibers can be changed due to melting and agglomeration of lignin at high temperature above 140 °C. Consequently, the steam explosion enhances the accessibility of cellulose to cellulases, and improves its enzymatic digestibility due to the removal of hemicelluloses and re-distribution of lignin [76]. However, generation of further degraded products such as organic acids, furfural (FF), and 5-hydroxymethyl-2-furfural (HMF) is a major drawback of the steam explosion due to its high pretreatment severity since they are known as strong inhibitors for fermentative microorganisms [77]. However, steam explosion has an advantage of causing less environmental problems due to no added chemicals and reduced operational and capital costs. Moreover, the process is feasible to be developed at the industrial scale.

4-2. Hydrothermal Pretreatment

Without adding any chemicals, lignocellulosic biomass can be pretreated by only either steam or hot water, during which the biomass recalcitrance can be reduced by changes in chemical composition and physical structure. The simplest method is called hydrothermal pretreatment, also termed as liquid hot water (LHW), hydrothermolysis, or autohydrolysis. The hydrothermal pretreatment can be classified into physical method, when only heat is applied, but it is also categorized in thermochemical pretreatments due to acid-catalyzed hydrolysis of hemicelluloses resulting from acetic acid liberated from acetylated hemicelluloses at higher temperature. Operating and capital expense for biomass conversion applied

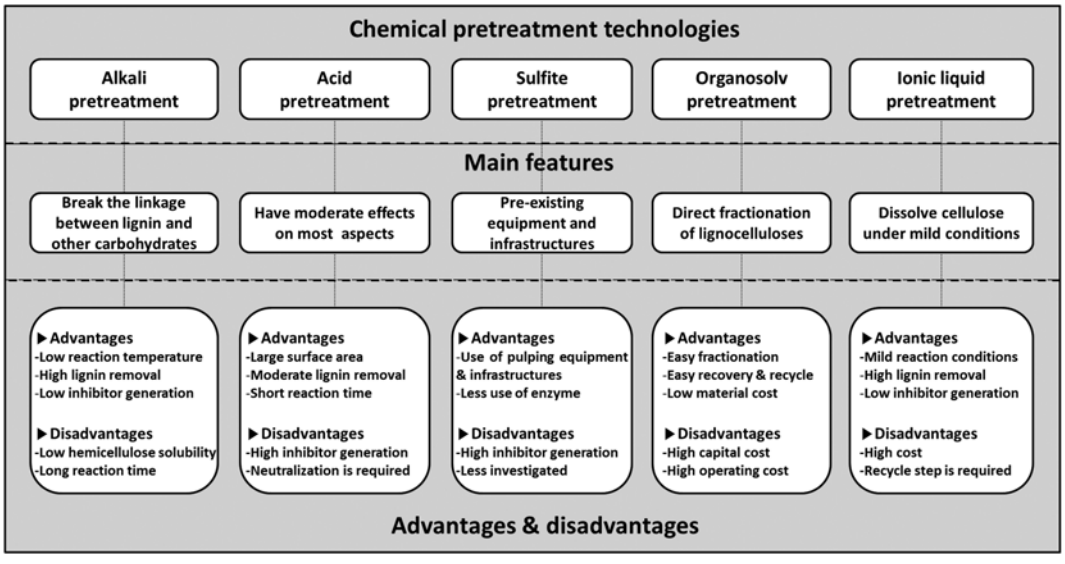

Fig. 2. Comparison of different technologies for chemical pretreatment of lignocellulosic biomass.

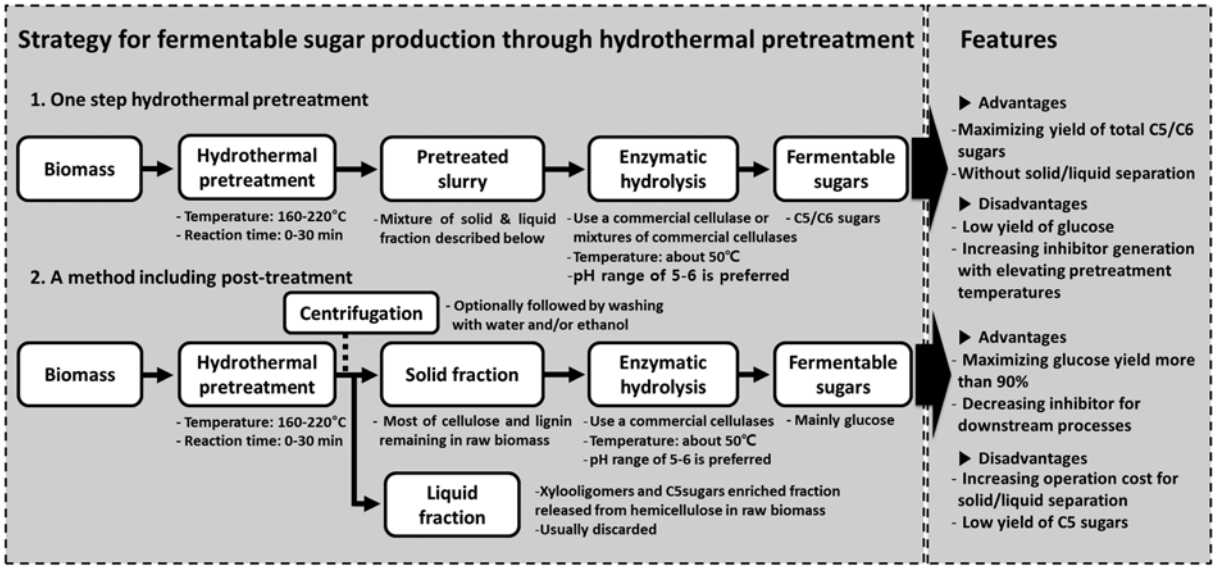

Fig. 3. A schematic illustration of hydrothermal pretreatment process.

in hydrothermal pretreatment can be lower compared to other pretreatment processes since no chemicals are used in hydrothermal pretreatment. After biomass pretreatment, in addition, there is no need to recover/recycle used chemicals and require complicated conditioning for subsequent enzymatic hydrolysis. However, unfortunately, hydrothermal pretreatment does not extract as much hemicelluloses as possible over acid pretreatment resulting in the requirement of higher enzyme dosage, which directly affects increase of operational costs. Alternately, it can be possible to remove up to 80% of total hemicelluloses, when the lignocellulosic biomass is subjected to higher temperatures around 200 °C. At such high pretreatment severity, however, a significant part of hemicelluloses is also further degraded to inhibitory products for subsequent enzymatic hydrolysis and microbial fermentation (Fig. 3). To remove large extent of hemicelluloses without their further degradation, one study has proposed a multi-stage hydrothermal pretreatment strategy to obtain high yield of sugars [78]. This process can be summarized as follows. In its first stage, hemicelluloses are hydrolyzed to either monomeric sugars or oligosaccharides at lower temperatures ranging of 170-180 °C, and then separated from pretreated slurry. After that, the remaining solid enriched cellulose and lignin is treated at higher temperatures between 190-200 °C to enhance susceptibility of cellulose to cellulases (Fig. 3). Processing and operating costs of this two-step pretreatment can be rather high, since solid-liquid separation is needed and higher energy is required to achieve favorable sugar yields. A variety of studies have shown that hydrothermal pretreatment is effective in converting herbaceous biomasses, such as switchgrass, sunflower stalks and wheat straw to fermentable sugars [79-83]. It has been reported that different yields of glucose and hemicellulosic sugars from the sunflower stalks were obtained depending on pretreatment temperatures ranging from 160 to 220 °C for 30 min. At 180 °C, hemicellulosic sugars were maximized up to yield of 74.6% of their theoretical yields, whereas glucose yield was just 67.0% of its theoretical yield [81]. When the stalks were applied to hydrothermal pretreatment at 200 °C, the highest glucose yield reaching 76.8% of its theoretical yield was obtained, but the yield of hemicellulosic sugars was fairly reduced to 19.7% of their theoretical yield due to their further degradation. However, the resulting glucose yield was noticeably increased to 83.0% of its theoretical yield, when pretreated solid separated from pretreated slurry at 180 °C was subjected to enzymatic hydrolysis. In case of washing pretreated solid obtained at above-mentioned way with hot water and then ethanol, its resulting sugar yield reached 89.9% of theoretical yield. Given the results, in order to achieve favorable sugar yield, there is no need to remove large extent of hemicelluloses from the biomass through being conducted at high pretreatment severity. Its enzymatic digestibility can be enhanced by post-treatment such as solid-liquid separation of pretreated slurry and washing pretreated solid resulting in favorable sugar yields from hydrothermal pretreatment, which is comparable to that from dilute acid pretreatment.

4-3. Ammonia Fiber Explosion (AFEX)

AFEX process can disrupt the biomass recalcitrance by combining physical effect and chemical reaction. In the AFEX process, lignocellulosic biomass is loaded to a pressurized reactor and then filled by anhydrous liquid ammonia with a solid and liquid ratio of 1 to 1-2. This slurry is heated to 60-150 °C, and then saturated for a period of time under high pressure below 200 psi, after which the pressure is suddenly reduced to get explosive expansion of the ammonia gas [84]. During the AFEX process the biomass recalcitrance can be broken down by induced shearing forces and chemical reactions affecting fibrillation, swelling, deacetylation, and cleavage of lignin-carbohydrates ester linkages, which leads to make cellulose more accessible to cellulases. Unlike steam explosion, the AFEX pretreatment produces only solid fractions of which chemical compositions are similar to those of untreated biomass. It is interesting to note that enzymatic digestibility of AFEX-treated biomass attained more than 90% of theoretical yields from carbohydrates resent in the biomass despite of little removal of hemicelluloses and lignin during the pretreatment. This result could be related to altered lig-

nin structure during the AFEX, resulting in reducing irreversible adsorption of cellulases on the lignin [85-87]. The AFEX process has an advantage of reducing operational costs, because ammonia recovery is relatively more feasible than other chemicals used in various pretreatments due to its high volatility, which implies that the ammonia can be used for pretreating biomass on a commercial scale [87].

5. Combined Pretreatment Technologies

To produce fermentable sugars from the lignocellulosic biomass at low severity and enzyme dosage, to date a number of studies have been performed. The combined chemical pretreatment and subsequent mechanical refining as post-treatment has an advantage of improving enzymatic digestibility at lower enzyme dosage and pretreatment severity, which leads to lower generation of hemicelluloses and lignin derived-inhibitors for subsequent enzymatic hydrolysis and microbial fermentation [88]. Mechanical refining on a commercial scale has commonly been used in the pulp and paper industry to enhance fibrillation of the pulp for the improved papermaking properties [89]. A number of studies have shown that cellulose digestibility could be significantly increased after post-refining treatment even at lower enzyme loading and pretreatment severity could be enhanced compared with that of untreated biomass [88-90]. According to Ertas et al. [90], the highest total sugar yield of 72.3% of total carbohydrates in wheat straw (76.8% of sugar yield from cellulose and 64.8% of sugar yield from hemicelluloses) was obtained from pretreated biomass by hydrothermal treatment at 180 °C for 20 min, followed by post-refining treatment and its enzymatic hydrolysis with 4 FPU of enzyme loading. At such pretreatment condition without the post-treatment, total sugar yield was only 57.2% [90]. In addition, some studies have suggested that pretreatment severity for pretreating the lignocellulosic biomass can influence mechanical refining efficiency in respect of its enzymatic digestibility compared to that of the unrefined biomass [88]. If the biomass is pretreated at higher severity, its enzymatic digestibility is not noticeably increased after mechanical refining; however, favorable sugar yield with less generating inhibitors can be obtained despite lower enzyme loading, when the lignocellulosic biomass is pretreated at mild conditions, followed by mechanical refining. Therefore, it is important for maximizing the refining efficiency in respect of sugar yield and inhibitor generation to select the suitable pretreatment severity.

BIOREFINERY INDUSTRY PRODUCTS

1. Biofuels

Ethanol is one of the oldest and main products obtained by the biorefinery process. It is produced by fermentation using yeasts and other microbial strains. Brazil is the leading country in the bioethanol industry. The National Alcohol Program (PróAlcool) created by the Brazilian government has already implemented 25% ethanol combined gasoline. This has reduced their import of 550 million barrels of oil and their CO_2 emissions by 110 million tons. Currently, 44% of their energy is renewable with 13.5% originating from sugarcane. Bio-ethanol from sugarcane has already been commercialized in Brazil and 80% of the vehicles run on bio-ethanol [91]. However, bioethanol converted from sugarcane, sugar beets, and sorghum may compete with the food supply and result in various social problems related to food shortages; thus, use of other feedstocks for ethically reasonable and cost-efficient biofuel production has been prompted. Sugarcane bagasse, the porous residue of cane stalks, has also been used as a renewable feedstock and requires a lower investment, infrastructure, and energy supply. Due to the complexity of the structure of lignocellulosic biomass, pretreatments such as steam explosion have been employed to increase susceptibility of the plant polysaccharides to acid or enzymatic attack [91]. Other pretreatments such as an alkaline-washing pretreatment to extract the lignin prior to enzymatic hydrolysis and IL-pretreatment have also been developed. Binder and Raines reported

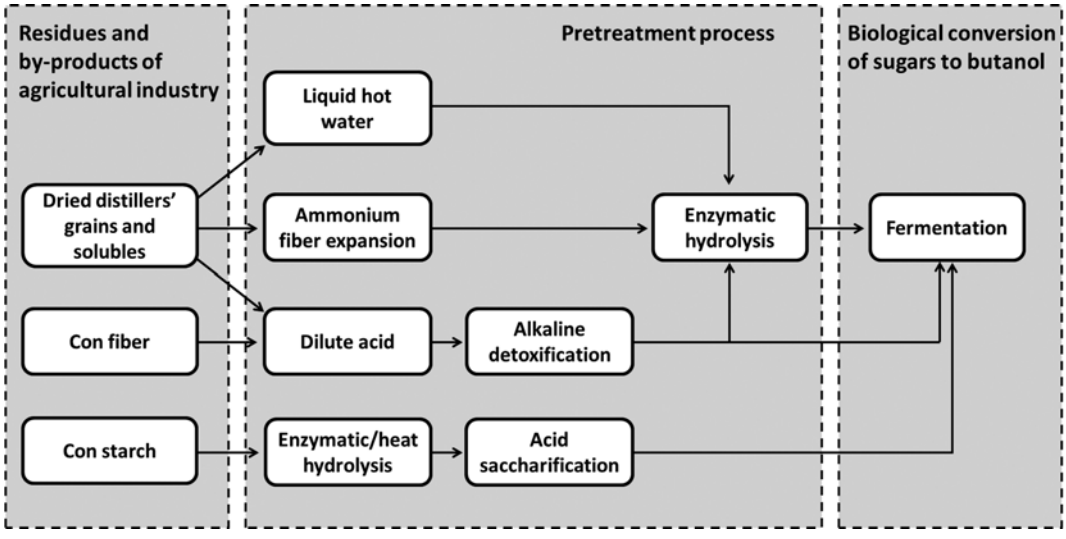

Fig. 4. A schematic illustration of actual process of butanol production by *C. beijerinckii* BA101 using several raw materials. Lignocellulosic biomass, dried distillers' grains and solubles (DDGS) were pretreated by three different methods and the hydrolysates possessing sugars such as glucose, galactose, mannose, xylose, and arabinose were used as fermentation feedstock to produce butanol by *C. beijerinckii* BA101. Corn fiber and corn starch were pretreated with different methods to prepare fermentation feedstock for butanol production.

a 79% ethanol yield using *E. coli* KO11 from IL-pretreated corn stover [92].

Butanol, a four carbon alcohol with a higher energy density than ethanol, has also been produced by fermenting various feedstocks [93,94]. In the early 20th century, industrial scale production of butanol by fermentation was carried out to satisfy increased need for synthetic rubber and solvents during World War I, but, this industry based on fermentative production of butanol has declined due to its higher production costs compared with those of chemical industry based on petroleum. In recent years, butanol has regained both academic and industrial interest since it has several advantages over ethanol as biofuel and shortages of fossil fuels and other natural resources are certain in the near future. Various kinds of feedstocks have been employed to produce butanol by fermentation (Fig. 4). *Clostridium beijerinckii* BA101 produced 13.4 g/*l* butanol using liquefied corn starch, which was 98% of the control using glucose [95]. In addition, all sugars constituting lignocellulosic biomass such as glucose, xylose, mannose, arabinose, galactose, and cellobiose could be consumed by this strain to produce butanol [96]. TetraVitae Bioscience has the license to use this strain for commercial purposes.

On the other hand, since large amounts of inhibitors of fermentation such as phenols, aromatic compounds, aliphatic acids, furan aldehydes, and inorganic compounds are produced during acid-pretreatment and hydrolysis of lignocellulosic biomass, detoxification is often required to use fermentable sugars obtained from lignocellulosic biomass in the fermentation of microorganisms for the production of desired products without much growth inhibition. For example, use of acid-treated corn fiber hydrolysates as a carbon source resulted in a significant decrease in butanol titer. However, by removing the inhibitors using XAD-4 resin and $Ca(OH)_2$, titers could reach 51% and 86% of control values, respectively [96, 97]. Enzymatic treatment can also be used to reduce the inhibitory effects of hydrolysates. The addition of a fungal peroxidase from *Coprinus cinereus*, accompanied by acidification and precipitation, could remove most of the phenolic compounds from the fermentation medium and thus, an equal or slightly higher amount of butanol was produced by *C. beijerinckii* [98].

2,3-Butanediol (2,3-BD), a potential biofuel as well as a platform chemical for many applications, is one of the most promising products in the bioindustry [99]. It can be produced from renewable resources by microorganisms such as *Klebsiella pneumonia*, *K. oxytoca*, *Enterobacter aerogenes*, and *Serratia marcescens* [100]. It was reported that 84.03 g/L 2,3-BD was produced in 40 h with the yield of 0.29 (g/g substrate) using *K. pneumonia* from Jerusalem artichoke tubers by a fed-batch simultaneous saccharification and fermentation [101]. Corncob molasses, which is a by-product in xylitol production, can also be used to produce a high concentration of 2,3-BD. 78.9 g/L 2,3-BD was produced using *K. pneumonia* from the feedstock by a fed-batch fermentation [102]. In addition, it has recently been reported that *E. aerogenes* produced 98.69 g/L 2,3-BD from sugarcane molasses with the yield of 0.366 (g/g substrate) [103].

These biofuels above can also be produced from marine algae. Extracts from *Laminaria hyperborean*, a brown algae with high levels of mannitol and laminaran, yielded 0.43 (g ethanol/g substrate) in batch cultures [104]. In another study, *Zymobacter palmae* was used to ferment mannitol extracted from brown algae into ethanol with a yield of 0.38 (g/g substrate) [105]. Marine microalgae can also be used as carbon source in ethanol production. After extracting oils from a microalga, *Dunaliella tertiolecta*, the remnant was further treated by acidic and enzymatic saccharification to yield fermentable sugars, and about 7g/L of ethanol was obtained from the hydrolysate with the yield of 0.14 (g/g residual biomass) and 0.44 (g/g glucose) [106]. Both mannitol and glucose extracted from the brown algae *Saccharina* spp. were also applied for the production of butanol by *Clostridium acetobutylicum* ATCC824 [107]. Butanol and total solvent weight yields from the brown algae were 0.12 and 0.16 (g/g substrate), respectively [107]. In both cases, the seaweed was extracted by using hot water (65 °C) for 1 hr at pH 2.0 [107]. Besides monosaccharides and disaccharides, both of which can be readily utilized for the fermentation of microorganisms, the storage carbohydrate of brown algae such as Laminarin showed a lag phase reflecting the need to be depolymerized prior to fermentation as described in one study using different macroalgae for the production of ethanol [108]. Acid-pretreated and enzymatically hydrolyzed brown seaweed *L. japonica* resulted in a higher sugar yield of 0.4 (g/g substrate) and was examined for 2,3-BD production. The sugar fraction obtained by acid pretreatment and enzymatic saccharification of the brown algae was converted into 2,3-BD by engineered *E. coli* with the titer of 14.1 g/*l* and the yield of 0.32 (g/g substrate) [109].

2. Biochemicals and Biopolymers

Besides biofuels, bioplastic is one of the other predominant products in the biorefinery market. Bioplastics can be classified into three major categories by their manufacturing processes: (a) biopolymers such as polyhydroxyalkanoate (PHA) and poly-γ-glutamic acid (PGA) of which monomers are produced by metabolic pathways in microorganisms and then processed further to polymers in their hosts; (b) biopolymers such as polylactide (PLA), poly-butylene-succinate (PBS), poly-trimethylene-terephthalate (PTT), and Nylon 4, whose monomers are produced by microorganisms and then are processed in vitro to polymers by chemical catalytic reactions; (c) biopolymers such as Nylon 5.10 and Nylon 6.10 whose feedstocks are biomass, but their production is solely composed of chemical processes.

Among these, bioplastics of the second category is dominating in current bioplastic markets and PLA is one of the leading products of this category. The PLA manufacturing process developed by NatureWorks LLC is composed of biological production of lactic acid, chemical cyclization of lactic acid into lactide, and ring opening polymerization (ROP) of lactide to synthesize PLA [110]. The first step often employs lactic acid bacteria as producers of the precursor using corn as feedstock. The market price of PLA is comparable to that of commodity plastics produced by petroleum-based processes mainly due to the low cost of feedstock and the technological as well as cost effectiveness of the latter chemical steps. However, due to social and ethical issues of using food crops, non-edible raw materials, such as cassava flower, paper sludge, rice bran, wheat bran, sugarcane bagasse, vine shoot, and oil palm trunk have also been investigated for lactic acid production [111-117]. One of the impressing results involves an optically pure L-lactic acid produc-

tion by genetically engineered *L. paracasei* using hydrothermally pretreated oil palm trunk as fermentation feedstock [112]. The host strain produced L-lactic acid with a 45.7% of theoretical maximum lactic acid yield (TLY) from hydrolyzed solid fraction of hydrothermally treated oil palm trunk while it produced L-lactic acid with an 89.5% of TLY from hydrolyzed whole slurry of hydrothermally treated raw material. Such a high yield production of L-lactic acid could be possible mainly because of the mild conditions of hydrothermal pretreatment. However, at present, none of the above processes can surpass corn-derived lactic acid production in terms of product titer, product yield, productivity, and production cost.

Besides lactic acid, production of several biochemicals that can be used as biopolyamide monomers has been examined using pure glucose as a carbon source, the predominant monosaccharide after enzymatic hydrolysis of biomass, which includes cadaverine, adipic acid, γ-aminobutyric acid (GABA), and 5-aminovaleric acid (5-AVA) [118-123]. As PLA is chemically synthesized using microbial fermentation derived lactic acid, polyamides can also be synthesized using these monomers that are prepared from fermentation processes. One of the impressive results involves the production of Nylon 5.10 that was synthesized by a condensation polymerization of cadaverine and sebacic acid. In this process, which is patented by BASF SE (Ludwigshafen, Germany), a hyper-cadaverine producing *Corynebacterium glutamicum* was constructed by the metabolic engineering of a previously constructed lysine producer [119,124]. The resulting strain could convert renewable carbon sources to the nylon monomer with a high product titer and molar yield [119]. The final product had superior mechanical properties compared to commercial Nylon 6 and Nylon 6.6 and well compounded with glass fiber for further enhancement of the material properties [119]. The hybrid process for the synthesis of bio-based nylon from fermentation-derived nylon monomers is illustrated on Fig. 5. In addition, 5-AVA can also be produced by employing the lysine synthetic pathway. It was first demonstrated that recombinant *E. coli* expressing the *Pseudomonas putida davB* and *davA* genes encoding lysine 2-monooxygenase and delta-aminovaleramidase, respectively, could successfully produce 5-AVA using lysine added to the culture medium as a direct precursor [122]. Although only 0.5 g/L of 5-AVA was produced by recombinant *E. coli* from glucose, lysine-hyper producing *C. glutamicum* has been suggested as strong candidate host for the high level production of this building block [122,125]. In addition, a process of Nylon 5.6 synthesis, which is composed of microbial 5-AVA production, isolation, purification, and following polymerization process, has recently been successfully demonstrated [125]. Another compelling example is GABA, of which the microbial synthetic pathway involves glutamate, which is one of the other dominant products in commercial amino acid market. Therefore, it seems to have a great potential in terms of production capacity as well as process consistency since *C. glutamicum*, potential host for GABA production, has already been employed for the production of glutamate and lysine by fermentation with an annual production of more than 1.5 million tons. Recently, 38.6 g/L of GABA was produced by fed-batch fermentation of recombinant *C. glutamicum* ATCC13032 expressing a mutant *E. coli* glutamate decarboxylase active in wide range of pH. Since the wild-type *C. glutamicum* ATCC13032 is not a glutamate-hyper producer used in commercial glutamate production process, it is expected to enhance GABA production even more by employing an industrial strain. However, despite the potential capacity of *C. glutamicum* as an industrial producer of biofuels, biochemical, and bioplastic monomers, relatively few studies have been conducted to search appropriate raw materials from biomass for *C. glutamicum* compared to the above-described microbial host strains widely used in biorefinery.

Beside microbial fermentation, sugars can also be converted to building blocks by catalytic reactions. One of the representative examples is 5-hydroxymethylfurfural (HMF), which can be used as a precursor for production of various chemicals. It was demonstrated that 2,5-dimethylfuran, 2,5-furandicarboxylic acid, levulinic acid, 2-hydroxymethylfuran, and 2,5-dimethyltetrahydrofuran could be efficiently produced by an efficient glucose conversion process [126]. Fructose can also be used as a substrate for production of HMF via acid-catalyzed reactions. A high HMF yield of 79% was obtained by a dehydration reaction of fructose in IL media containing

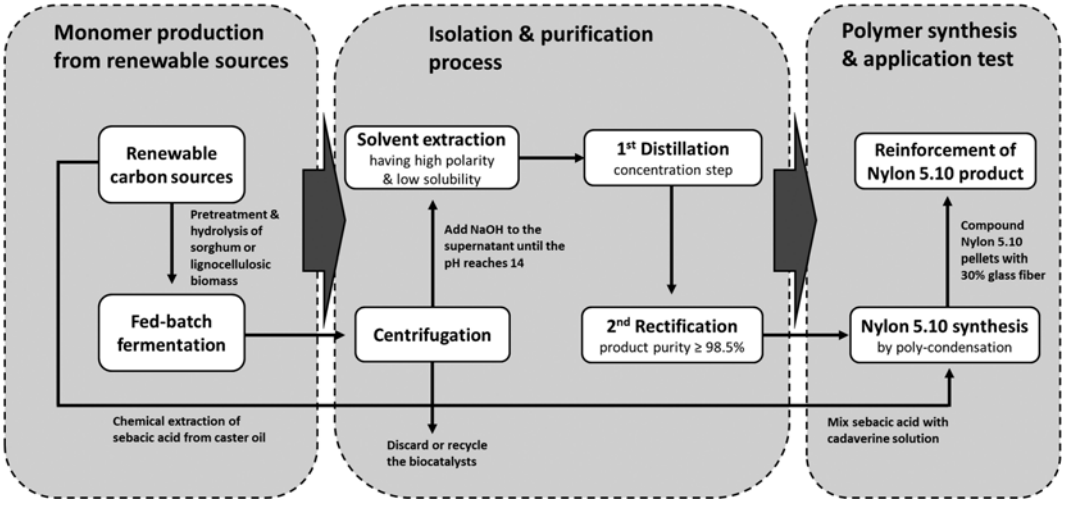

Fig. 5. A schematic illustration of the hybrid process: from the preparation of bio-nylon precursors to the synthesis of Nylon 5.10.

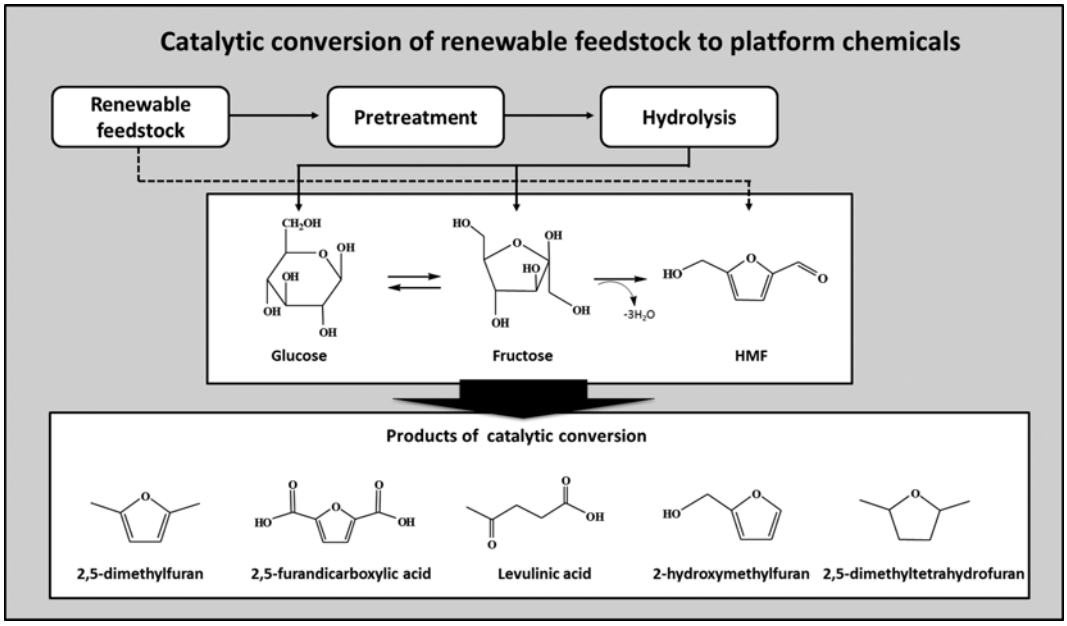

Fig. 6. A schematic illustration of catalytic conversion process of renewable feedstock into HMF and its derivatives.

10.0 mmol of 1-butyl-3-methylimidozolium chloride ([Bmim]Cl) and 0.20 mmol of $NbCl_5$ [127]. In addition, Binder and Raines converted untreated lignocellulosic biomass such as milled corn stover, sieved corn stover, and pine sawdust into HMF with high conversion yields. From untreated corn stover, HMF was produced with a molar yield of 48%. Considering the HMF yields obtained from fine cellulose ranged from 4% to 54% according to their reaction conditions, corn stover seems to be a promising raw material for the production of HMF [128].

CONCLUSIONS AND FUTURE PERSPECTIVES

Although selecting the appropriate biomass and pretreatment process is the key step that determines production cost, there is still no ideal pretreatment or process condition. Biomass pretreatment still remains a bottleneck for effectively utilizing energy crops to produce biofuels and other bio-based chemicals. In fact, much money, time, and manpower have been expended searching for suitable biomass sources that converted to sugars for fermentation and to develop an effective pretreatment process. Nevertheless, pretreatment technologies must be studied on a fundamental scale to determine their impact on each biomass source to maintain a balance between higher yield of sugars and lower further degradation of carbohydrates to inhibitory products for enzymes and microorganisms. Knowledge of the biomass composition, including chemical structure, will help predict which type of pretreatment method is appropriate. In addition, economics as well as process dynamics should be carefully analyzed to apply these pretreatment technologies on an industrial scale. In conclusion, an integrated biorefinery process still seems to be a promising route, but it will involve a coordinated effort from pretreatment chemistry and fermentation product profiles. Directly converting pretreated biomass to high-value and platform chemicals by chemical catalysis is also worthy of in-depth study.

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