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# Production of Biofuels from Cellulose of Woody Biomass

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Pedram Fatehi

Additional information is available at the end of the chapter

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## 1. Introduction

Today, fossil resources supply approximately 86% of energy and 96% of organic chemicals used in the world [1]. The continuous depletion of petroleum fuel is one of the prime concerns these days. Other concerns associated with the large-scale utilization of fossil fuels are availability, global warming and uneven geographic distribution [2,3]. Also, the global population is expected to increase by approximately 3 billion people by 2050, which substantially increases the need for fuels. One estimate indicated that the world energy consumption would increase by 35% over the next 20 years in order to meet the growing demand of industrialized countries and the rapid development of emerging economies [2]. As the fossil fuels are depleting in the coming years, new technologies should be developed in order to produce fuels from renewable biomass resources [4].

Currently, biomass accounts for 9.8% of the world's primary energy use annually, among which 30% is used in modern forms (e.g. liquid biofuel and steam), and 70% is used traditionally (combustion for domestic heating with the energy density of 15-20 MJ/kg) [2,4,5]. Biofuel is a type of fuel whose energy is derived from biomass. It includes solid biofuels such as wood pellets, wood cube and wood puck; liquid biofuels such as ethanol and butanol; and gaseous biofuel such as hydrogen. The world liquid biofuel production would increase from 1.9 million of barrels per day in 2010 to 5.9 million barrel per day in 2030 [2]. In the United States, expectation for the production of biofuels is 136 billion liter mandated by 2022, of which 61 million liter are made from cellulosic materials [6].

As described earlier, biomass has directly been used for producing heat and electricity. The power generation engines were designed so that they directly used biomass as energy source during World War II. However, this direct utilization has several major problems: if used as biofuel, the uneven geographical distribution of biomass necessitate its transportation, the bulky nature of biomass (low heat value) leads to costly and complicated

transportation systems; engines utilizing biomass usually possess a poor efficiency and high environmental impact (e.g. CO<sub>2</sub> emission). However, according to the first law of thermodynamics, any biological or chemical conversion of biomass to biofuel will consume energy, thus a part of the energy stored in biomass will be lost during the conversion and the biofuel (product) will ultimately have a lower energy than will biomass (raw material). There are several advantages for the conversion of biomass to biofuel that outweighs this deficiency: there is a large demand for liquid fuel (e.g. ethanol) in the transportation sector; the biofuel production process is more environmentally friendly (i.e. less CO<sub>2</sub> emission); the digested materials from biorefinery can be readily used as an excellent and sustainable fertilizer for cultivation and crops (a true recyclable process); the energy density (MJ/kg) of biofuel will be higher than that of biomass, and the common problem of combustion, e.g. fly ash disposal and super heater corrosion, can be eliminated via biofuel production [2,7,8].

One of the challenges of biofuel production is the difficulties in increasing the bulk density of the resource, while preserving its energy content [9]. The future biofuel should 1) have a high energy density on a mass; 2) be produced at yields near the stoichiometric maximum from a given biomass feed; 3) be compatible with existing fuel distribution infrastructures; 4) have a minimum impact on environment; and 5) not affect the global food supplies [9-11].

The first generation biofuels are presently produced from sugars, starches and vegetable oils, but these products have several issues: 1) their availability is limited by soil fertility and per-hectare yield; and 2) their contribution to savings of CO<sub>2</sub> emissions and fossil energy consumption are limited by high energy input for their cultivations and conversions [12-14]. However, lignocellulosic biomass seems to be more attractive because 1) it is the most widespread renewable source available on earth (overall chemical energy stored in biomass by plants is approximately 6-7 times of total human energy consumption annually [15]); 2) it is locally available in many countries; and more importantly 3) it does not compete with food or food industries [12]. However, the conversion of woody biomass to fermentable sugars is more difficult than that of agro-based biomass because of the presence of more hemicelluloses (not easily fermentable) and lignin as well as more condensed and crystallized structure of cellulose in woody biomass [9].

Current technologies to produce biofuel from cellulosic materials involve gasification, pyrolysis/liquefaction and hydrolysis of biomass. This book chapter excludes 1) the studies on the gasification and pyrolysis/liquefaction of biomass (as an intact raw material) for biofuel production; and 2) the studies on the production of fuel additives, e.g. levulinic acid and furfural [16] and the studies on the production of biodiesel [17-22]. Instead, recent advancements and challenges associated with the production of ethanol, butanol, hydrogen and new furan-based biofuel from cellulosic biomass will be discussed.

In order to produce biofuel from cellulose of biomass, the lignocellulosic biomass should be first disassembled to facilitate the isolation of cellulose from other constituents, i.e. lignin and hemicelluloses. Subsequently, cellulose macromolecules should be depolymerized, as depolymerization significantly improves the chemical and biological conversions of cellulose to biofuel. Then, the depolymerized cellulose, i.e. glucose, should be converted to

biofuel via biological treatments, and finally the biofuel should be purified. Additionally, biomass should be deoxygenated as the presence of oxygen reduces the heat content of molecules and creates high polarity, which impairs its blending with existing fossil fuels [12].

## 2. Pretreatment of biomass

The complex structure of lignocellulosic biomass makes its utilization in biofuel production difficult. Lignocellulosic raw materials are generally composed of 40-50% cellulose, 25-35% hemicelluloses and 15-20% lignin [8]. A pretreatment stage is necessary to dissociate the plant cell wall in order to improve the accessibility of chemicals and/or microorganisms to cellulose for possible conversions [23]. The pretreatment processes target the removal of lignin, which improves the digestibility of cellulose in the following hydrolysis process [24]. Table 1 lists various pretreatment processes of woody biomass conducted in the past in order to improve the performance of fermentation processes in producing biofuels.

Pretreatment type	Example
Physical pretreatment	Ball milling, Irradiation
Physicochemical pretreatment	Steam explosion, hot water pretreatment
Chemical pretreatment	Acid, alkali, solvent
Biological	Fungi
Enzyme	Cellulase

**Table 1.** Various pretreatment processes of lignocellulosic feedstock [14]

Physical pretreatment consists of mechanical disruption of lignocelluloses, which is an environmentally friendly process. This process increases the surface area of biomass and decreases the crystallinity of cellulose, but it does not cause an expensive mass loss [25]. Irradiation using gamma rays, electron rays and microwaves are other physical methods to break the structure of lignocelluloses. Microwave irradiation has been applied in many fields including food drying chemical synthesis and extraction [14].

Physicochemical pretreatment is another approach to separate the lignocelluloses of woody materials. Hydrothermal treatment, such as hot water pretreatment and steam explosion, is a suitable method particularly prior to enzyme hydrolysis. Hot water pretreatment process is conducted under pressure at an elevated temperature of 230-240 °C for 15 min to maintain water in liquid form, which produces less inhibitory compounds (e.g. furfural) compared to steam explosion method [26,27]. However, the viscosity of the spent liquor produced in this method is rather high, which makes its handling process challenging. The steam explosion is practically applied in industry via steaming biomass at an elevated temperature, e.g. 170 °C [28-30]. To limit the production of inhibitors, process conditions should be precisely adjusted. Steam explosion has different subcategories, such as ammonia fiber explosion and acid-explosion, in which acid or ammonia is also added to the system during the steaming process.

The chemical pretreatment of cellulosic biomass includes oxidizing hydrolysis, acid or alkaline hydrolysis and solvent extraction. In this context, lime treatment (e.g. treatment of woody biomass at 180 °C with lime solutions) has been considered as an effective chemical pretreatment method, because of its low cost and wide use in agro- and wood-based processes [4,27,31-34]. Pretreatment with dilute acid at intermediate temperatures (e.g. 160 °C) is usually considered the most cost-effective method to loosen the cell wall matrix via degrading the hemicelluloses of biomass [27,35].

Biological pretreatment, such as fungal, is milder in its operational conditions than physical or chemical pretreatment. The oxidative biodegradation of lignin by white-rot fungi has been widely studied in the past [36]. The main advantages of biological pretreatment are low energy input, no chemical requirement, mild environmental conditions and environmentally friendly working manner [25]. However, the biological pretreatment processes usually need a long retention time and have a low yield. They are also sensitive to the process conditions such as temperature and pH. Enzymatic pretreatment of biomass has been comprehensively studied in the past and will be discussed in a separate section.

### 3. Hydrolysis

Generally, microorganisms have poor cellulose/biomass digestibility and a limited efficiency in producing biofuels. Hydrolysis has been commonly applied in industrial scales to improve the efficiency of microorganisms in producing biofuels prior to fermentation, which relies on the decomposition of polysaccharides to monosaccharides [37].

#### 3.1. Enzyme hydrolysis

In this process, enzyme is used for decomposing polysaccharides into monosaccharides. Microorganisms that usually produce enzyme are fungal species, such as *hypocerea jecornia*, *trichoderma reesei*, and bacteria species, such as *clostridium thermocellum*, *cellulomonas flavigena*. Three main enzymes hydrolyzing cellulose to glucose are *cellulase*, *1-4- β-D-endoglucanase* and *1-4- β-D-cellobiohydrolase* [38]. Process parameters, e.g. pH, temperature, time, significantly affect the performance of enzyme hydrolysis. Furthermore, porosity and crystallinity and the lignin content of biomass seem to significantly influence the efficiency of this process [25].

Equation 1 describes the enzymatic hydrolysis model of cellulose to glucose [39]:

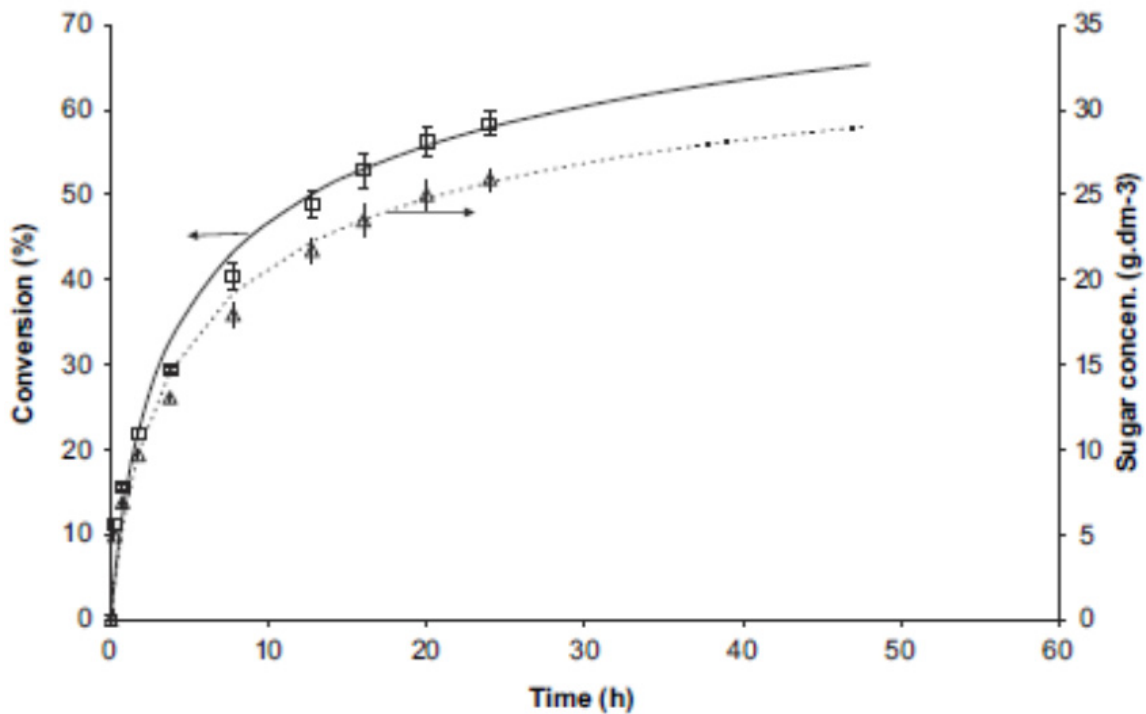
$$X = 1 - \left[ \frac{K_e + e_0}{K_e(K_a e_0 t + 1) + e_0} \right]^b \quad (1)$$

where  $e_0$  is the initial enzyme concentration (g/l),  $X$  is conversion efficiency (<1),  $t$  is the reaction time (h),  $k_a$  is the enzyme deactivation constant (g/l.h),  $b = k_e k_a k_1$  is the fitted constant (dimensionless),  $k_e$  is the adsorption equilibrium constant (g/l), and  $k_1$  is the rate constant of sugar formation (1/h). When  $e_0 \rightarrow \infty$ ,  $X$  converges to a constant at constant time according to equation 2:

$$X = 1 - \left[ \frac{1}{K_e K_a t + 1} \right]^b \quad (2)$$

and when  $t \rightarrow \infty$ ,  $X$  converges to a maximum conversion of 1.

Figure 1 shows the results of one study on the enzyme hydrolysis of pre-steamed corn Stover at 50 °C and pH 4.8, the conversion of celluloses to reducing sugar was 60% after 48h. Fitting the experimental data of Figure 1 into equation 1 resulted in  $k_e$ ,  $k_a$ ,  $k_1$ , and  $b$  of 0.9975 (g/l), 0.9837 (1/g.h), 0.2843 (1/h), and 0.2897, respectively [39].



**Figure 1.** The conversion of cellulose and the concentration of reducing sugars as a function of time in the enzymatic pretreatment. □, Δ experimental points; solid and dash lines denote the model (Eq. 1) values [39].

Enzymatic hydrolysis has several advantages compared with acid hydrolysis such as a lower equipment cost and higher glucose yield without sugar degrading products or by-products [24].

### 3.2. Acid hydrolysis

This method has been extensively applied to convert oligomeric sugars to monomeric sugars in the past [40-43]. It has a short process time (i.e. less than 1 h) and produces a higher sugar yield (>85%), but operates at a relatively high temperature, e.g. >120 °C (compared to enzyme hydrolysis). However, acid hydrolysis may result in the production of undesirable by-products that should be eliminated prior to fermentation processes. These detoxification processes are generally costly and complicated [25,40-42].

#### 4. Detoxification process

Natural occurring and process-induced compounds may retard the fermentation processes for producing biofuels, which complicates the fermentation process [42-46]. These inhibitors are phenols, acetic acid, furfural, metal ions and lignosulfonates, and can chemically or biologically be removed from hydrolysates prior to fermentation processes.

Boiling and overliming have been extensively used in different studies to reduce the concentration of the inhibitors [44-46]. Boiling was proposed to reduce the concentration of volatile components, e.g. furfural, and overliming was proposed to create some insoluble inorganic salts that adsorb inhibitors [47]. Alternatively, the inhibitors can be removed by employing the concept of adsorption and flocculation phenomena. In this regard, commercial adsorbents, e.g. activated carbons, fillers, e.g. calcium carbonate or lime, or ion exchange resins may be suitable choices. The adsorption/flocculation processes could effectively remove these inhibitors and research in improving the performance of this process is on-going [28,30,48-50].

Alternatively, the inhibitors can be removed from hydrolysates via biological treatments. For example, a mutant yeast, *S. cerevisiae* YGSCD 308.3, was applied to reduce the acetic acid content of the ammonia-based hardwood hydrolysate in one study [51]. This yeast grows on acetic acid, but not on xylose, glucose, mannose and fructose. This detoxification process resulted in an ethanol production (fermentation was conducted at a temperature of 30 °C, and 300 rpm for 24 h) with a 73% yield of that of the maximum theoretical value in a laboratory scale. However, the presence of acetic acid did not allow ethanol production in the control sample of this study [51].

#### 5. Ethanol production

As of January 2008, 136 ethanol plants in the US produced 7.5 billion gallons of ethanol annually, and this number is expected to increase by 13.3 billion gallons/year via constructing additional 62 plants [4]. The current biofuel market is largely dominated by ethanol (90% of the world biofuel production) [2]. However, most of ethanol produced today comes from starch (as in maize grains) or sucrose (from sugarcane), i.e. first generation biofuel. Lignocellulosic biomass, on the other hand, represents more abundant feedstock for ethanol production, i.e. the second generation biofuel [4]. However, the cost of producing lignocellulosic ethanol could be almost double of that of corn-derived ethanol [2]. In this context, the US department of Energy (DOE) has committed over \$ 1 billion dollars toward a realization of a 2012 goal of making lignocellulosic ethanol at a competitive cost of \$1.33 per gallon [52].

The production of ethanol from biomass has been criticized in the past since a large amount of CO<sub>2</sub> is produced (released) as the by-product of this fermentation process. However, one study showed that, if softwood (unspecified species and fermentation conditions) biomass were considered as raw material, and ethanol were produced from cellulose and hemicelluloses and FT-diesel from lignin, this integrated process could lead to 54% of mass

conversion efficiency, 67% of carbon conservation efficiency, but more interestingly, 88% of energy conversion efficiency [12]. In other words, the majority of mass, but just 12% of energy, would be lost in ethanol production process. Consequently, ethanol production process would definitely improve the energy density of biomass, which is a critical factor of biofuel. This analysis depicted that it was extremely crucial to widen the assessments beyond the sole mass balance to obtain a complete understanding of biofuel production [12].

### 5.1. Process alternatives

The biological processes of ethanol production usually involve hydrolysis, which breaks cellulose to glucose, and fermentation to convert glucose to ethanol. Ethanol can be produced via separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF). The SHF facilitates the operation of hydrolysis and fermentation processes under separate optimized conditions. Previous studies on pre-steamed agro-based raw materials (corn Stover) revealed that the yield (ethanol produced per unit mass of dried feedstock g/g) of SHF was higher than that of SSF [53]. Also, SHF has a faster hydrolysis rate than does SSF under optimized conditions [11,39]. In SSF process, cellulose is hydrolyzed to glucose by cellulase, while yeast spontaneously ferments glucose to ethanol [54]. The SSF usually has a higher productivity (ethanol produced per unit mass of dried feedstock per unit time, g/g.h) than does SHF, since SSF has a shorter operating time [39]. Other advantages of SSF over SHF include less inhibition of enzymes and a longer time of enzymatic hydrolysis. Usually, fermentation temperature and the presence of ethanol are not at optimized conditions for cellulase activities in the SSF process, which leads to poor enzyme performance. To overcome this difficulty, several approaches were followed in the past, which are demonstrated as follows:

1. Enzyme immobilization onto a solid support was proposed to improve the enzyme activity at non-optimal conditions (e.g. SSF). In one study, dissolved cellulase (500  $\mu$ l, in 10 mM acetate buffer, pH 4.8 with 0.01 % (w/v) sodium azide) was immobilized on Aerosol OX-50 silica (40 nm average diameter particle, 1.4 m<sup>2</sup> surface area), and the SSF was carried out using this coated silica [54]. The results showed that the ethanol yields of the SSF process containing immobilized enzyme were 2.3 and 2.1 times higher than that of the SSF process containing enzyme in solutions at pH 4.8 and 5.3, respectively. The higher ethanol yield of the immobilized enzyme process was likely due to higher glucose yields as a result of increased enzymatic stability at the non-optimal enzyme conditions required for the SSF [54]. However, this process results in a higher ethanol yield under the optimum conditions of fermentation rather than that of enzyme hydrolysis. This is because 1) the optimum conditions for the immobilized enzyme reaction may not coincide with those for the enzyme in solutions; 2) although the same mass of enzyme can be used in the solution and immobilized systems, it does not mean that the same amount of enzyme is available to participate in the hydrolysis reactions; and 3) enzyme immobilization results in a random orientation of adsorbed enzyme on the silica substrates, and this leads to some inactive enzyme due to buried or inaccessible active sites [54].



2. Semi-simultaneous saccharification and fermentation (SSSF) is another approach to produce ethanol. In this method, the hydrolysis process is applied under optimized conditions, which breaks down cellulose to glucose, and subsequently fermentation is conducted on the product of hydrolysis without removing the hydrolystate. In other words, it is an operating mode between the SHF and SSF, thus it has the advantages of both SHF and SSF (a higher productivity and yield). In one study on microcrystalline cellulose (Avicel PH101), three alternative processes were carried out to produce ethanol: 24h hydrolysis+48h SSF (called SSSF), 72 h SSF, and 24 h hydrolysis+48h fermentation (SHF) [39]. The hydrolysis process was conducted under the conditions of 50 °C, pH 4.8 and 2 g Novozymes enzyme, while the fermentation was performed under the conditions of 36 °C, pH 4.8 and 300 rpm using *S. cerevisiae*. The results showed that the optimal ethanol productivity for SSSF, SSF and SHF were 0.222, 0.194, and 0.176 g/l.h, respectively. The corresponding maximum ethanol concentration was 16, 14 and 12.6 g/l with equivalent theoretical ethanol yields of 70.5%, 61.8%, and 56.1%, respectively [39].

## 5.2. Theory

The theoretical ethanol yield (Y) can be calculated using equation 3 [39]:

$$Y = \frac{0.9E}{0.511C_0} 100\% \quad (3)$$

where E is the final ethanol concentration (g/l) and C<sub>0</sub> is the initial cellulose concentration (g/l). Also, the fermentation efficiency (e<sub>f</sub>), from sugar to ethanol, can be determined using equation 4:

$$e_f = \frac{E}{0.511G_h} 100\% \quad (4)$$

where G<sub>h</sub> is the glucose concentration after hydrolysis (g/l). When glucan in cellulose is completely converted into glucose (C<sub>0</sub>=0.9 G<sub>h</sub>). The theoretical yield of SSF yield is equal to fermentation efficiency [39].

## 5.3. Microorganism for ethanol production

Ethanol production via fermentation has been the subject of several research activities. In this regard, many yeasts and bacteria have been introduced or modified and their ethanol production efficiencies have been assessed.

### 5.3.1. Bacteria

*Escherichia coli* can consume hexoses and pentoses for ethanol production. However, *E. coli* is less robust against several factors including pH, salt concentration and temperature. It also exhibits a low ethanol (<35 g/l) and butanol tolerance (< 20g/l) [51]. *Z. mobilis* has also been applied in ethanol production, has a tolerance of up to 120 g/l ethanol, and its ethanol production yield approaches 97% of the maximum theoretical value under optimized conditions. However, it can only ferment glucose, fructose and sucrose (hexoses), and it has a low tolerance to acetic acid [55,56]. *Clostridia* have also been applied in ethanol production

under optimized temperature of 35-37 °C at a pH range of 6 and 9, which can ferment hexoses and pentoses [57]. *Corynebacterium glutamicum* has also been applied for ethanol production, but it cannot ferment pentoses, unlike *E. coli*. [58].

### 5.3.2. Yeast

Hexoses can effectively be converted to ethanol with a high yield (0.4-0.51 g ethanol/ g glucose) and a high productivity (up to 1 g/l.h) by *Saccharomyces cerevisiae* or re-combinant *S. cerevisiae* [53,55]. *S. cerevisiae* is the best known microorganism used for fermenting glucose to ethanol, but it cannot ferment pentoses. In this respect, the fermentation rate of xylose is 3-12 times lower than that of glucose by *S. cerevisiae* [45]. *Kluyveromyces* is another thermotolerant species with up to 98% of the maximum theoretical ethanol yield at a temperature above 40 °C, but it cannot accommodate pentose [59]. *Hansenula polymorpha* is another thermotolerant species with an optimized temperature of 37 °C, and its fermentation operation is possible up to a temperature of 48 °C. *Kluyveromyces* and *H. polymorpha* are suitable for SSF processes [60]. *P. stipitis* is another naturally fermenting pentose and hexose. It can be used for SSF set-up even though its optimized growth temperature is around 30 °C [53,55].

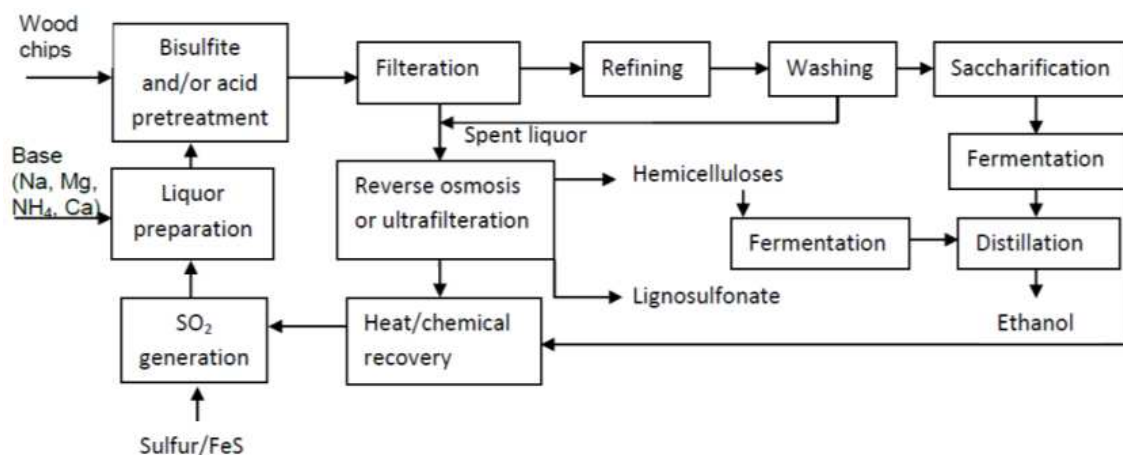
## 5.4. Ethanol recovery

The ethanol production is coincided with yeast cell growth in fermentation, hence the yeast is a by-product of the process. Pure yeast is a value-added product of the process and can inevitably decrease the net cost of the process [53]. Currently, centrifugation is applied to separate yeast cells from ethanol, which is an expensive process with a high energy demand. Yeast cell immobilization technologies using inert carries or chemicals have also been applied in ethanol industry for separating ethanol from cells [53]. Alternatively, yeast flocculation process was introduced in the 1980s and commercialized in 2005 in China and comprehensively used in brewing industry. It involves lectin-like proteins and selectively binds the mannose residues of cell wall of adjacent yeast cell. In this flocculation process, calcium ions are needed and the flocculation occurs spontaneously [61-63]. Upon formation, the flocs would either sediment (large yeast) or rise to the surface (ale yeasts). This flocculation process has the advantages of 1) allowing greater yeast cell biomass concentrations because of no inert carrier; 2) being a simpler and more economically competitive process and 3) fostering the yeast cell viability because the continuous renewal of cells resulting from breaking up of relatively large flocs. The yeast flocs can be purged from the fermenter maintaining the biomass concentration inside the fermenter at specified levels [64]. Different configurations of immobilization process are available for industry: airlift, single packed column, two-stage packed column and CO<sub>2</sub> suspended bed [64].

## 5.5. SPORL and dilute acid ethanol production processes

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) has been developed by the U.S. Forest Service, Forest Products Laboratory and the University of

Wisconsin-Madison as a promising biorefinery process to produce ethanol from cellulosic materials [32,65,66]. The SPORL process has a short chemical pretreatment at a high temperature to remove recalcitrance of the substrate without a significant delignification, and a disk refiner to increase the surface area, which is necessary for the sugar dissolution in the latter stages for fermentation. The potential products of the SPORL process are ethanol, hemicellulosic sugars and lignosulfonate [67]. Figure 2 shows the process diagram of the SPORL process. As can be seen, the wood chips are pretreated with bisulfite and/or sulfuric acid at a temperature of 160-190 °C, a pH of 2-5, and liquid/wood ratio of 2-3 for 10-30 min. The bisulfite charge is 1-3% and 6-9% for hardwood and softwood species, respectively, and acid ranges between 1 and 2% [68]. The solid substrates of the chemical treatment are then fibrillized using a mechanical disk refiner. The chemical pretreatment has a direct effect on the refining stage of the SPORL process. Subsequently, the solid substrate is enzymatically hydrolyzed, fermented, and distilled to produce ethanol [67]. Hemicelluloses are also isolated from the spent liquor and fermented to ethanol, while lignosulfonate is a by-product of this process. Softwoods species have usually poor digestibility in enzymatic saccharification [65]. The SPORL process is particularly effective in improving the enzymatic saccharification efficiency of softwoods.



**Figure 2.** Process flow diagram of the SPORL or dilute acid treatment [67].

Alternatively, ethanol can be produced by pretreating biomass with sulfuric acid, and subsequent mechanical pulping (refining) of the pretreated biomass. This process is called dilute acid process. However, a high temperature and a very low pH of this process impart serious equipment corrosion problems [70].

It was reported that the SPORL pretreatment was more efficient than the dilute acid pretreatment for recovering sugars from the solid substrate and spent liquor. Inhibitors were more in the spent liquor of the dilute acid pretreatment, but the concentration of lignin was higher in the spent liquor of the SPORL pretreatment. Due to the lower inhibitors presented in the spent liquor, the ethanol production would be facilitated with the SPORL system. The ethanol production from the substrate and spent liquor of pine species under various SPORL pretreatment conditions at 180 °C for 25 min and then fermenting via using *S. cerevisiae* (ATCC 200062) at 32 °C for 72 h at 100 rpm are listed in Table 2 [70].

Acid charge, %	Bisulfate charge, %	Initial pH	Substrate ethanol <sup>1</sup>	Substrate ethanol efficiency <sup>2</sup>	Hydrosate ethanol <sup>1</sup>	Hydrolysate ethanol efficiency <sup>2</sup>	Total ethanol yield
2.21	4	1.8	136.7	73.7	52.8	65.1	189.5(49.2)
2.21	8	1.9	209.4	83.8	66.9	94.2	276.3(71.7)
1.4	8	2.3	193.2	76.4	36.3	70.4	229.5(59.6)
0	8	4.2	165.6	69.6	1.7	11.3	166.7(43.3)

<sup>1</sup>:l/ton wood; <sup>2</sup>:percentage of the theoretical yield

**Table 2.** Ethanol production in the SPORL system of pine species under various conditions [70].

As can be seen, by increasing the bisulfite concentration from 4% to 8%, the ethanol production and efficiency were increased for both the substrate and spent liquor, resulting in 21% increase in the total ethanol production. It is also noticeable that the ethanol production from the substrate or spent liquor was reduced by reducing the acid charge (increasing pH) of the pretreatment.

## 6. Butanol production

Although ethanol has been considered as a promising biofuel, it has several drawbacks: the heat value of ethanol is 27 MJ/kg, while that of FT-diesel is 42.7 MJ/kg implying that ethanol contain a much lower energy density compared with diesel fuel used in automobile industry [2,12]; it has also a high water solubility that prevents it from being an ideal biofuel, and high concentration ethanol blends can cause corrosion of some metallic components in tanks and deterioration of rubbers and plastic used in car engines [2]. Consequently, the incentives for obtaining a better alternative biofuel are high.

The industrial synthesis of biobutanol was commenced during 1912-1914 by acetone-butanol- ethanol (ABE) fermentation of molasses and cereal grains using *clostridium acetobutylicum* [71]. However, butanol was primarily used as a solvent for the production of other chemicals prior to 2005. Butanol has similar energy density and polarity to those of gasoline [71]. It has an adequate blending ability with gasoline and compatibility to combustion engines [72]. It can be shipped through existing fuel pipelines, whereas ethanol must be transported via rail and truck [73,74]. It has octane-improving power and low volatility (six times less than the volatility of ethanol). These novel properties made butanol a promising biofuel [75]. The economics of butanol fermentation is favorable even with the present technology [76]. However, the capital cost of butanol fermentation is presently higher, but its production cost is less, than that of petrochemical process to produce butanol [77]. Despite steadily growing production, its market remains tight and its price high due to low investment and hefty demand in coming years [76].

### 6.1. Butanol fermentation process

To produce butanol via fermentation as the second generation biofuel, cellulose should be initially converted to glucose, as most of the butanol-fermenting microorganisms can digest

glucose and not cellulose. Similar to ethanol production, the enzymatic hydrolysis of polysaccharides to monosaccharides and then fermentation to biosolvents were carried out in the past [78]. The drawbacks of this process is its energy intensity, which makes its commercialization costly [78,79].

The production of butanol from cellulose mainly relies on the application of *clostridia* species (*C. acetobutylicum*, *C. beijerinckii*, *C. pasteurianum*). *Clostridia* acetone-butanol-ethanol (ABE) fermentation from carbohydrates used to be the largest biotechnological process second to yeast ethanol fermentation and the largest process ever conducted under sterile conditions [72,76]. *Clostridia* species have several advantages: their thermophilic nature permits their utilization at a high temperature of 60 °C (facilitates sterilization process), they can digest pentoses, and more interestingly they can ferment cellulose, which implies that cellulose hydrolysis to glucose and glucose fermentation can be proceeded spontaneously. Butanol can only be generated if the cells enter sporulation process, but this process discourages cell growth. Thus, balancing cell growth and sporulation timing is a key factor influencing the carbon flow towards cell growth and butanol generation [74]. However, these species have some disadvantages including the low solvent resistance, comparative difficulty in genetic modification, and increased energetic demand for cellulase production under anaerobic environments [80].

In the ABE process, the coproduction of acetone, butanol and ethanol causes a poor selectivity with respect to butanol production. The theoretical mass and energy yields of ABE fermentation are 37% and 94%, respectively [81]. It was reported that the substrate costs account for 60% of the total production cost, and the butanol production will not be feasible if the fermentation yield is less than 25% [80]. The best results reported for the ABE process were 8.2 g/l acetone, 2.2 g/l ethanol and 17.6 g/l butanol [82].

## 6.2. Production improvement

The production of butanol faces some challenges: 1) selection of sustainable biomass 2) low production rate, 3) constrains executed on butanol inhibition and 4) high product recovery costs [72,83]. Different strategies can be performed to improve the production of butanol via fermentation as described in the following sections.

### 6.2.1. Eliminating inhibitors

The phenolic compounds of cellulosic materials inhibit the butanol fermentation process (similar that of ethanol fermentation process) [78]. In one study, the majority of inhibitory compounds were phenolic compounds, while furfural and hydroxymethyl furfural (HMF) did not affect the cell growth and ABE fermentation [84]. It was reported that, during the initial growth phase of cells, other by-products were also produced such as acetic acid and butyric acid, which inhibited the butanol production [84]. In one study, the presence of 1 g/l ferulic acid and vanillin acid reduced the cell growth by 70% and 56%, respectively [84]. The choice of detoxification method would depend on the compositions of hydrolysates and the species of fermenting microorganism [84]. Previously, lime treatment [85], evaporation, adsorption using ion exchange resins and activated carbon were used prior to fermentation

as detoxification methods for butanol production [84]. However, the detoxification efficiency depends on the chemical structure of inhibitors [84]. Other inhibitors of the ABE process are substrate inhibition, salt concentration, presence of dead cells, low water activity, O<sub>2</sub> diffusion, macromolecules accumulation and nutrient deficiency [86].

### 6.2.2. Removal of butanol

*Clostridia* species are known to be solventogenic in producing acetone, butanol, and ethanol, but are still subjected to negative inhibition by their own products [72,74]. The hurdles are being resolved using genetic engineering techniques, metabolic engineering strategies and integrated continuous fermentation processes [72]. In this context, one study showed that the presence of butanol at a concentration of higher than 7.4 g/l impaired the cell growth [87,88]. In this case, butanol may penetrate the cytoplasmic membrane and disrupt several physicochemical characteristics of the cells [72]. On the contrary, the *Clostridium* BOH3 species showed the advantage of high resistance against butanol (up to 16 g/l) [74].

In the literature, the simultaneous production and the removal of butanol was carried out in order to maintain a low butanol concentration in the fermentation medium (broth) using liquid-liquid extraction, adsorption, perstraction, reverse osmosis, pre-evaporation and gas stripping [89], among which gas stripping has received great attentions. Gas stripping offers several advantages including feasibility and simplicity of the process, reduction in butanol concentration without affecting culture, concentration of nutrients and reaction intermediates [86]. Furthermore, a membrane separation with super critical extraction may be a feasible method in butanol removal in future [72].

### 6.2.3. Modifying microorganism

One alternative to increase the butanol production was reported to be the genetic engineering for strain improvement with insertion of butanol producing gene of *Clostridia* in high butanol tolerant organisms. In this context, the genetic manipulating of *Clostridia* was reported to decrease its sensitivity to the presence of butanol, which eventually increased the butanol concentration up to 17.8 g/l in the fermentation medium [87]. Also, research on aerobic producing butanol using genetically engineered organics like, *E. coli*, are being attempted [72]. The strain improvement is effective in improving the yield, but has marginal influence on the economics.

## 6.3. Process configuration

The fermentation of glucose to butanol can be conducted in batch or continuous process. Generally, continuous butanol processes, (free cells, immobilized cells, and cell recycling) are more economical over batch processes. Other advantages are the reduction in sterilization and inoculation times and the superior productivity. In free cell continuous fermentation, cells are free to move within the fermentation broth due to agitation or air lifting. This maintains the microbial cells and nutrients in the suspension and helps in promoting mass transfer [72]. In

immobilized cell fermentation, cells are stationary, which have the advantages of the long survival time of cells in solventogenic phase. In one study, immobilized cell fermentation using *C. acetobutylicum* produced 20% higher butanol yield than did free cell fermentation [90]. However, the scale up process using immobilized technology seems to have technical problems due to excessive cell growth in the packed beds and blockage [85]. Recycling technique using a membrane technology (e.g. filter) after the fermentation has also been attempted in the literature and the results showed 10 times higher cell concentration and 6 times higher butanol production compared with conventional continuous process using *C. saccharoperbutylacetonicum* [85]. On the contrary, batch process has a less capital cost and contamination problem (sterilization) in fermentation, and is more flexible.

## 7. Hydrogen production

Hydrogen has been considered as one of the major energy carriers in future due to its high energy content and its capability to overcome the air pollution and global warming [91]. Renewable carbohydrates (e.g. cellulose) are suitable raw materials for hydrogen production, i.e. second generation biofuel, because they are less expensive than other hydrogen carriers, e.g. hydrocarbons, biodiesel, methanol and ethanol [3,37]. Glucose is widely used substrate for hydrogen production [37]. Today, the production cost of hydrogen from renewable biomass is appealing (\$ 60/dry ton or \$ 3.6 per GJ) [15]. The most important energy application of hydrogen is transportation, especially for light-duty vehicles [3]. However, the large scale implementation of the hydrogen economy has some obstacles: sustainable hydrogen production, high density hydrogen storage, hydrogen distribution infrastructure, fuel cell cost and life time, and safety concerns [3]. Thus, new technologies and strategies should be developed to make the hydrogen production more economically attractive and industrially feasible [14].

### 7.1. Hydrogen production processes

Table 3 lists various pathways to produce hydrogen from glucose [3]. As can be seen, the theoretical and practical yields of hydrogen production via chemical catalytic reactions, i.e. gasification, pyrolysis and hydrolysis (accompanied by aqueous phase reforming (APR)), of glucose are in a similar range. The gasification is conducted at a high temperature of 1000 K in the presence of oxygen and water. Pyrolysis is carried out at a high temperature but in the absence of oxygen. The main advantage of APR over gasification is the lower extent of undesirable decomposition reaction [3]. The APR is carried out at a lower temperature (400-550 K) and a medium pressure (50-70 bar) [3]. The water medium of this process promotes the occurrence of the hydrogen production reaction. Although the chemical catalytic reaction seem to have considerably higher hydrogen production yields, their prerequisite high energy input and poor selectivity toward hydrogen production are barriers in their industrial implementations.

However, the biological conversion of cellulose to hydrogen is performed at much lower temperatures, which implies that the energy input of this process is much lower than that of

chemical catalytic processes. In contrast, the theoretical and practical yields of hydrogen production via this method are rather low (see dark anaerobic fermentation in Table 3) [30,92,93]. In principle, up to 12 mol of hydrogen can be produced per mole of glucose and water via fermentation. However, natural microorganisms produce as much as 4 mol of hydrogen/ mol of glucose along with 1 mol of acetate [94]. The current yield of hydrogen production in cellulose fermentation ranges from 1 to 2 mol H<sub>2</sub>/mole of hexose sugar [95]. To increase the hydrogen yield, a bioelectrochemically-assisted microbial fuel reactor was reported to convert 2 mol of acetate to up to 8 mol of hydrogen with the aid of electricity [96]. In this respect, the overall production yield of hydrogen increased to 9 mol per mol of glucose [96]. However, this process also needs electricity input and has not been studied in large scales.

Method	Theoretical yield, %	Practical yield, %	Energy efficiency, %
Dark fermentation (DF)	4	1-3.2	10-30
DF+ electricity-assisted microbial fuel cell	12	9	75
Ethanol fermentation/partial oxidation reforming	10	9	60
Gasification	12	2-8	35-50
Pyrolysis	12	2.5-8	30-50
Hydrolysis+ aqueous reforming	12	6-8	30-50
Synthetic pathway biotransformation	12	12	122

**Table 3.** Methods for converting glucose to hydrogen as biofuel [3].

Synthetic pathway biotransformation is a new biocatalytic technology based on the application of enzymes to convert cellulose to hydrogen. This process is much simpler than biological treatments. The biotransformation of carbohydrates to hydrogen by cell-free synthetic pathway (enzymes) has numerous advantages: high production yield (12 H<sub>2</sub>/glucose unit), 100% selectivity, high energy conversion efficiency (122% based on combustion energy), high purity hydrogen generated, mild reaction conditions, low cost bioreactor and no toxicity hazards. In one study using 2 mM cellubiose concentration as the substrate, the overall yields of hydrogen and CO<sub>2</sub> were 11.2 and 5.64 mol per mol of anhydroglucose unit corresponding to 93% and 94% of the theoretical yields, respectively [97]. Furthermore, these enzymatic reactions are reversible, thus the removal of products favors the unidirectional reaction towards the desired products. One study on the biotransformation of starch and water revealed that these reactions were spontaneous and endothermic ( $\Delta G_0 = -49.8$  kJ/mol,  $\Delta H_0 = +598$  kJ/mol). Thus, these reactions are driven by entropy gain rather than enthalpy loss [3]. Such entropy-driven reactions can generate more output chemical energy in the form of hydrogen than input energy in the form of polysaccharides [97]. This is very interesting as most of the chemical conversions of cellulose/glucose to biofuel are exothermic. In other words, the output biofuel has less



energy than do raw materials, i.e. cellulose/glucose. However, this method currently has a low hydrogen production rate [3]. Research in this area is on-going and no concrete conclusion has been made yet.

## 7.2. Biological production of hydrogen

The biological production of hydrogen has received great attentions due to its potential as an inexhaustible, low-cost and renewable source of clean energy [91]. The photosynthetic production of hydrogen basically uses CO<sub>2</sub> and water via direct photolysis. The yield of this process is high, but there is a shortage of photobioreactors on large scales, which constrains its investigation in laboratory scales [98]. Compared to photosynthesis, anaerobic hydrogen production process is more feasible (less expensive), has higher rates and thus has been widely studied [99]. This process seems to be closer to be implemented in commercial scales.

Several breakthroughs have been made in understanding the fundamentals of hydrogen production including the isolation of microbial strains with a high hydrogen production capacity and the optimization of the microbial fermentation process [100,101]. The performance of anaerobic fermentation depends on a number of factors, e.g. temperature, pH, alkalinity oxidation-reduction potential, particle size of lignocellulosic materials, substrate content and inoculum source [91,102]. It was reported that the optimized pH for producing hydrogen in a dark fermentation is between 5.5 and 6.7 [102]. However, there are contradictory reports about the influence of ethanol on hydrogen production in dark fermentation processes in that some studies reported a possible competition between ethanol and hydrogen production [103-105], while others reported a high hydrogen production accompanied by a high ethanol production [106,107]. In another study, the addition of ethanol to the growth medium at the initiation of the fermentation process resulted in 54% H<sub>2</sub> and 25% acetate increases, respectively, using *C. Thermocellum* bacteria [108].

In addition to hydrogen, organic acids are produced in dark fermentation. To achieve adequate overall energy efficiency, the energy stored in the organic acids produced in the dark fermentation processes should also be utilized. This can be conducted via combining the concepts of dark fermentation and anaerobic digestion [109].

On the contrary, dark fermentation has some drawbacks: 1) poor yield per substrate in the conversion of biomass to H<sub>2</sub> by microbial fermentation; 2) sensitivity to end-product accumulation; and 3) high environmental and economic costs of biomass production to generate fermentable substrate [13,110-112].

## 7.3. Microorganisms to produce hydrogen

Thermophilic cellulosic bacteria promote their greater operating temperature to produce hydrogen, which facilitate biomass pretreatment, maximize enzymatic reaction rates, and favor the equilibrium point of H<sub>2</sub> in direction of H<sub>2</sub> production [13,14]. *Clostridium thermocellum* and *caldicellulosiruptor saccharolyticus* have been the main focus of hydrogen production research [95]. *Caldicellulosiruptor saccharolyticus* is a gram-positive and extremely thermophilic, has been

reported to produce hydrogen from cellulose even at a high temperature of 70 °C, and is the most promising candidate for large scales hydrogen production [98,113]. Hydrogen has been reported to be produced from pentoses via using thermophile *Caldicellulosiruptor saccharolyticus* with a high yield of 334.7 ml H<sub>2</sub>/g of sugar accounting for 67% of maximum theoretical yield of 497.6 ml H<sub>2</sub>/g of sugar [37,114,115]. Furthermore, the optimum pH in the fermentation of cellulose to produce hydrogen via *C. Thermocellum* was between 7 and 7.2 [116].

In the literature, some mesophilic bacteria have been investigated for hydrogen production including *Clostridium cellulolyticum*, *Clostridium ellulovorans*, *Clostridium phytofermentans* and *Clostridium termitidis*. However, their low operating temperature (32-40 °C) introduces additional operating units to the hydrogen production process and increases the risks in process operations, which constrains the practical implementation of mesophilic bacteria in large industrial scales [3].

#### 7.4. Fermentation culture

Although the pure culture usually provides a high production efficiency, it is difficult to apply in industrial scales. In fact, the preparation of pure cultures in industrial scales is difficult and expensive, which will definitely affect the production cost of hydrogen. Instead, hydrogen can be produced with mixed cultures. The mixed culture has been claimed to be more effective in substrate conversion than pure culture [95].

However, mixed culture may encounter the drawbacks of the competition of substrates with non-hydrogen producing microbial population as well as the consumption of produced hydrogen by hydrogen consuming bacteria. One alternative to address this difficulty is to pretreat the mixed culture with base, heat and/or an anaerobic condition, which eliminates/inhibits the non-producing/consuming hydrogen bacteria [34,113,117]. Heat pretreatment is commonly used in anaerobic fermentation in order to improve the hydrogen production by activating spore-forming *clostridium* and inhibiting hydrogen consuming non-spore-forming bacteria. However, the heat pretreatment might inactivate hydrogen producing bacteria existing in natural feedstock [34,37]. The elucidation of anaerobic activated sludge microbial community utilizing monosaccharides will be an important foundation towards the industrialization of hydrogen production. In one study, a mixed microbial culture was obtained via the anaerobic activation of sludge in a continuous stirred-tank reactor (29 days of acclimatization) [118]. In this study, glucose had the highest specific hydrogen production rate (358 mL/g.g of mixed liquid volatile suspended solid) and conversion rate (82 mL/g glucose) among glucose, fructose, galactose and arabinose under the fermentation conditions of 35 °C and pH of 5 [34,118].

#### 7.5. Improving production efficiency

##### 7.5.1. Dark fermentation

The presence of hydrogen in fermentation reactors seems to affect the performance of dark fermentation process. It was reported that sparging the bioreactor with nitrogen would bring

the concentration of hydrogen at a low level in the fermentation. Alternatively, using hollow fiber silicone rubber membrane effectively reduced biogas partial pressure in the fermentation resulting 10% improvement in the rate and 15% increase in the yield of hydrogen production [119]. To improve the hydrogen production, some strategies were carried out in the literature involving bioprocess engineering and bioreactor design that maintains a neutral pH during fermentation and ensures the rapid removal of hydrogen and CO<sub>2</sub> from the aqueous phase [95].

### 7.5.2. Enzymatic treatment

Enzymes applied in the biotransformation of hydrogen are expensive. A combination of enzyme immobilization and thermo stable enzymes was reported to increase the life time of enzyme used for hydrogen production [120]. In one study, changing the process parameters (e.g. temperature, enzyme concentration, substrate concentration and metabolic channeling) in enzymatic reaction were reported to affect the hydrogen production rates [3]. A conservative estimation reported that the hydrogen production rates could increase to 23.6 H<sub>2</sub>/l/h using a high-cell density [121]. The over-expression of enzymes catalyzing reactions towards the desired product is another method to increase the hydrogen production. The heterologous gene expression of pyruvate decarboxylate and alcohol dehydrogenase from *Zymomonas mobilis* within *C. cellulolyticum* was shown to increase the hydrogen yield by 75%, while that of acetate and ethanol increased by 93% and 53%, respectively [122].

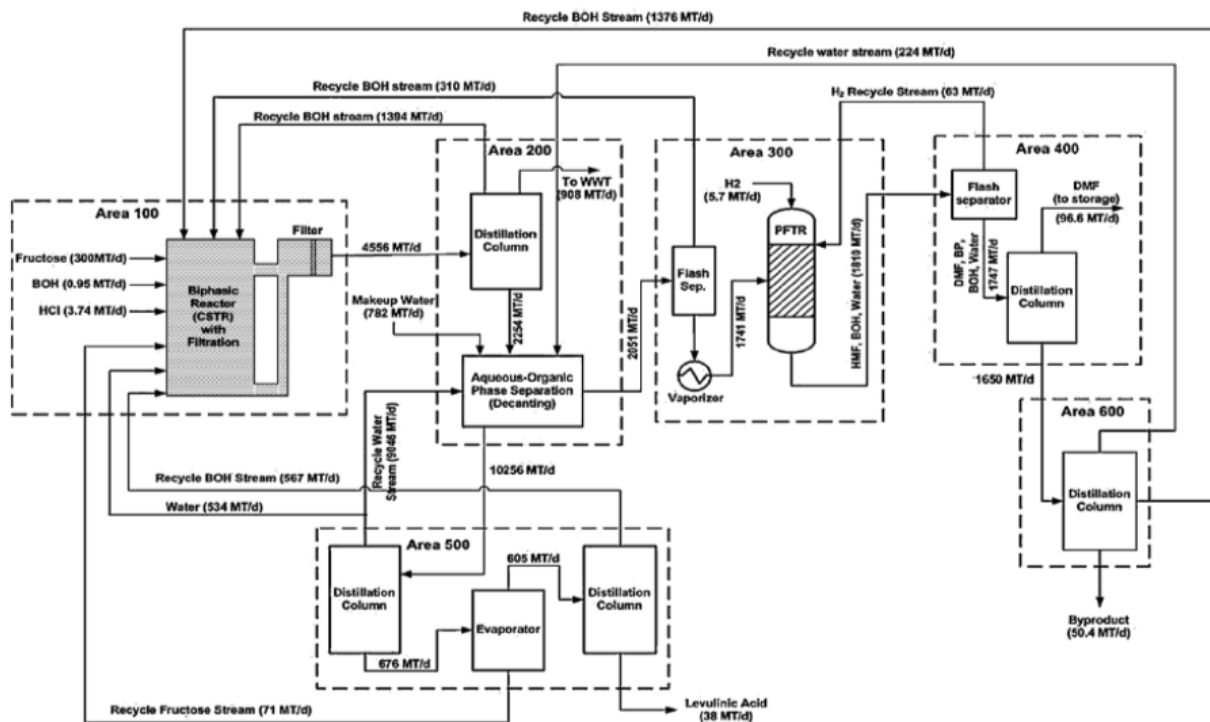
## 7.6. Process configuration

Similar to ethanol production, hydrogen production can be conducted in a one- or two-stage process. The simultaneous saccharification and fermentation process, i.e. one-stage process, is less expensive and more commercially feasible, but may be less efficient since the preferred conditions for cellulose degradation and dark fermentation could be significantly different [14]. An important parameter in this process is to choose a microorganism, such as *thermococcus kodakaensis*, *clostridium thermolacticum* and *clostridium thermocellum*, that has the capability to produce cellulolytic enzymes and hydrogen simultaneously [14,123]. In this process, the production rate and efficiency are limited by enzymatic saccharification. The two-stage hydrogen production process, on the other hand, is performed via cellulose hydrolysis in one stage and fermentation of the hexose in another stage [124]. This process might be more effective in terms of hydrogen yield, but it is more complicated.

## 8. Production of furan-based biofuel

Recently, a new second generation biofuel was introduced via the chemical conversion of cellulose. In this approach, hydroxymethyl furfural (HMF) is initially produced from cellulose, and HMF is subsequently converted to 2,5-dimethylfuran (DMF). DMF has an energy content of 31.5 MJ/l, which is similar to that of gasoline (35 MJ/l) and 40% greater than that of ethanol (23 MJ/l) [1]. DMF (bp 92-94 °C) is less volatile than ethanol (bp 78 °C), and is immiscible with water, which makes it an appealing liquid biofuel [1].

Figure 3 shows the process block diagram of DMF production from fructose. This process contains two main parts: 1) HMF production and its purification and 2) DMF production from HMF and its downstream purification.



**Figure 3.** DMF production from fructose [6].

Two-solvent catalyst system of butanol/water (at 180 °C) was proposed for producing HMF from fructose [1]. In this process, the conversion of glucose/fructose takes place in the aqueous phase (with HMF yield of 83%), in which HCl acts as a catalyst to convert fructose to HMF. Also, NaCl is added to the system and enhances the transportation of HMF from aqueous phase to organic phase (butanol), which prevents HMF from further degradation in the aqueous phase. Afterwards, the HMF is purified via various distillation/separation units and butanol is recycled to the HMF production reactor [1].

In another study, methyl isobutyl ketone (MIBK)/water system (in the presence of TiO<sub>2</sub> and 250 °C) resulted in 35% HMF yield from glucose [125,126]. In this respect, ionic liquids, e.g. 1-ethyl-3-methyl-imidazolium chloride ionic (in the presence of LiCl, CuCl or CrCl) showed a higher yield (>65%) at a temperature of 160 °C [127,128]. In another research, dimethyl sulfoxide (DMSO) was used as solvent to produce HMF from glucose with HMF yield of 53% [129]. However, these solvents are usually toxic and difficult to prepare, and their separation process from HMF is challenging [129]. These drawbacks will most likely limit their application in producing HMF in laboratory scales.

Alternatively, the hydrothermal conversion of cellulose to HMF (275-300 °C for less than 30 min) in homogenous systems in the presence of sulphuric acid resulted in a 20% HMF yield. However, this system under a neutral condition produced a lower yield (<16%), but a product (HMF) with a higher purity (50-60%) [130]. In another research, the yield of HMF

from fructose was 65% in the presence of phosphoric acid, which was conducted at 228 °C for less than 5 min [1]. The most promising and industrially attractive method to produce HMF was the acetic acid/water mixture (10 g/l acid at 200 °C for 20 min), which resulted in the production of HMF from fructose with 60% yield [131]. This process resulted in 53% HMF yield under the same conditions, but without acetic acid [131]. Alternatively, the hot compressed water treatment of cellulose at a temperature of 523 K for 5 min (pressure 2.5 MPa under nitrogen) produced 15% HMF, and the addition of 10% (wt.) TiO<sub>2</sub> to the system increased the yield to 28% [1]. This method is more feasible than other homogenous catalytic methods, as the catalyst in this system can be easily separated and thus recycled to the system [132]. Furthermore, the addition of acetone to water/TiO<sub>2</sub> system induced a higher HMF yield (35%). This process was conducted under atmospheric condition, which is more industrially attractive, but the presence of acetone in the system brings recycling issues and uncertainties at large scale applications [1].

The produced HMF in Figure 3 will be fed into a PFTR reactor, in which H<sub>2</sub> is added (in the presence of chromium II or copper-ruthenium-carbon catalysts) that is necessary for the conversion of HMF to DMF [6]. Finally, DMF is purified using several distillation/separation processes, and the organic solvent (butanol) is recycled to the system (Figure 3).

However, this process has several drawbacks: 1) it uses hydrogen which is a biofuel and currently expensive; 2) it uses butanol (another biofuel) as a solvent, which brings difficulties to its large scale implementations; 3) it uses NaCl to enhance the extraction of HMF from aqueous phase to organic butanol. NaCl introduces uncertainties in the downstream processes and its removal is cost intensive; 4) as described above, expensive chromium II or copper-ruthenium-carbon was used as a catalyst in the PFTR reactor [6]. It was reported that the production cost of DMF using this process is approximately 2 \$/l, which is not presently economical. The process optimization seemed to lower the production cost to approximately 1 \$/l, which is still high and not competitive with other appealing biofuels [6].

Alternatively, cellulose can be converted to 5-chloromethylfurfural (CMF) via heating cellulose in concentrated HCl at the presence of LiCl. Subsequently, the products should be extracted with 1,2-dichloroethane. This process yielded 71% CMF in one study [133]. The CMF was then converted to DMF and 5-ethoxymethylfurfural. This process yielded 84% isolated DMF, but the presence of LiCl is considered as one drawback of this system [133]. Alternatively, the CMF was converted to ethoxymethylfurfural (EMF) via stirring in ethanol solution. EMF has a boiling point of 235 °C, and energy density that is similar to energy density of gasoline and 40% higher than that of ethanol [133]. The EMF can also be used a biofuel, but this process seem to be complicated and faces with several technological challenges.

The most important parameters affecting the production cost of DMF via the aforementioned process are feedstock cost, production yield, by-product prices, catalyst cost, and total purchased equipment cost [6]. The productions of DMF and CMF are not feasible using current technologies. Although these chemicals were produced at laboratory

scales, their commercialization is under serious questions as their production processes require various expensive solvents that are not easily recycled. The separation and purification of final products from solvents are also costly.

## 9. Conclusions

The pretreatment of woody materials is an important step for producing biofuels via fermentation. The physicochemical pretreatment is the most promising approach to disassemble cellulosic biomass. Enzymatic hydrolysis is very selective in terms of decomposing cellulose chains, but its process conditions are not very industrially attractive. However, acid hydrolysis is fast with a high yield, but it produces some by-products. These by-products are inhibitors of downstream fermentation processes and hence should be removed from the process prior to fermentation. Adsorption and evaporation have been the most successful approaches to eliminate these inhibitors in detoxification stages prior to fermentation. *S. cerevisiae*, *C. acetobutylicum* and *C. thermocellum* are the most promising microorganisms for ethanol, butanol and hydrogen productions, respectively. Presently, the major challenges in the production of these biofuels are the rather low production yield of biofuels and the sensitivity of microorganisms to the presence of inhibitors and biofuels in the fermentation media. The productions of ethanol and butanol from woody biomass are close to be commercialized, but hydrogen production is still facing with difficulties in increasing the production rate. Although 2,5-dimethylfuran (DMF) has appealing properties and can be potentially used as biofuel, its production with present technologies is not economical and more industrially attractive processes should be developed in order to have a commercialized DMF process.

## Author details

Pedram Fatehi

*Chemical Engineering Department, Lakehead University, Thunder Bay, ON, Canada*

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