**บทความวิชาการ**

# Bioethanol Production from Cellulosic Material: A Review

กนกพร สังขรักษ์ [1*]
Kanokphorn Sangkharak [1*]

Nowadays, environmental awareness is a sensitive issue. Fossil fuels are implicated in the emission of greenhouse gases that pollute the environment. The utilization of bioethanol as an alternative fuel has attracted a lot of attention because it results to zero net carbon dioxide output into the atmosphere since it is recycled through photosynthesis [1, 2, 3]. Commercial bioethanol production mostly relies on fermentation of sucrose from sugarcane and mollases or waste originating from agricultural crops, households and industries, with wastepaper being a major component of these waste materials [4, 5, 6]. Paper manufacturing has been made a drastic impact on the environment as it requires 3.3 tons of trees and 0.4 tons of petroleum to make one ton of paper from virgin materials [7, 8]. Add to this the negative influence of dumped or burnt wastepaper materials and it becomes imperative to investigate ways to convert these used materials into useful products [8]. Wastepaper is known that cellulose, a major building block of paper products can be converted enzymatically to sugars including glucose [9, 10] and subsequently fermented to ethanol [11]. The efficient development of different wastepaper with cellulose as the major structural component would, however, rely on the extent of cellulose degradation into fermentable sugars. Bioconversion of wastepaper can be enhanced by pretreatments which include acid, alkali, ammonia and urea pretreatments, physical grinding and milling, steam explosion as well as combination alkali and heat treatment [9, 12, 13, 14, 15, 16].

To make the production of ethanol feasible for industrial application, research and development to increase the ethanol production with high yield is essential. Owing to the different purposes of paper products their structural composition differ, which produces varying susceptibilities for pretreatment process, a most important factor in the economics of the ethanol production process because it influences waste treatment, cellulose conversion rates and hydrolysis of hemicellulose sugars and their subsequent fermentation [9, 17, 18]. The specific goals of this study aim to understanding the production of bioethanol from cellulosic material through fermentation technology as well as their future direction.

---

[1] อาจารย์ หน่วยวิจัยชีวโมเลกุลพืช สาขาวิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยทักษิณ จังหวัดพัทลุง 93110

[*] Corresponding author: โทรศัพท์ / โทรสาร: 0-7460-9600 ต่อ 2355 e-mail: kanokphorn@tsu.ac.th

## INTRODUCTION

Over the 21st century, energy consumption has increased steadily as the social demands for energy, transportation, heating and industrial processes. Crude oil, non-renewable fuel has declined in worldwide since 2010 [19]. Campbell and Laherrere [20] predicted that annual global oil production would decrease from current 25 billion barrels to approximately 5 billion barrels in 2050. A decreasing of oil supply has been evidenced by increasing oil price, which during 2006 approached US$ 80 per barrel [21]. As well as the continued use of fossil fuels to meet the majority of the world's energy demand is threatened by increasing concentrations of $CO_2$ in the atmosphere and concerns over global warming [22, 23, 24]. Current method to control global warming issue focuses on reducing energy consumption and searching for renewable energy sources that reduce $CO_2$ emission. Bioethanol becomes a matter of widespread attention as a potential alternative to petroleum-derived fuels [24].

Unlike fossil fuels, ethanol is a renewable energy source produced though different feedstock and fermentation technology. Not only a renewable bio-based resource but bioethanol is also oxygenated (contains 35% oxygen) thereby provides the potential to reduce particulate and $NO_x$ emissions in compression-ignition engines. The advantage and disadvantage of bioethanol are shown in Table 1.

The advantage of bioethanol allows for a higher compression ratio, shorter burn time and leaner burn engine, which lead to theoretical benefit over gasoline and make bioethanol suitable for mixed fuel in the gasoline engine. Diesel-bioethanol blended fuel shows the great advantage in some properties such as high octane number, low cetane number and high heat of vaporization impede self-ignition in the diesel engine. So, ignition improver, glow plug, surface ignition, and pilot injection are applied to promote self-ignition [25]. Nowadays, bioethanol was applied as blend fuel in worldwide. The most popular blend for light-duty vehicles is known as E85, contains 85% bioethanol and 15% gasoline. However, in Thailand small amount of bioethanol is blended with gasoline in a mixture called gasohol E10 and E20 which contain 10% and 20% of bioethanol, respectively. The bioethanol-gasoline blend fuel have applied in some countries such as Brazil (E20, E25), The United State (E10), Canada (E10), Sweden (E5), India (E5), Australia (E10), China (E10), Columbia (E10),Peru (E10), Paraguay (E7) [24, 26]. Ethanol is produced by several of process, the most common of which is the fermentation of sugars. Nowadays the worldwide production of bioethanol is mainly produced from corn therefore the cost of ethanol is relatively high compared to

**Table 1** The advantage and disadvantage of bioethanol compared with fossil fuel.

| Advantage | Disadvantage |
|---|---|
| - high octane number | - lower energy density |
| - broader flammability limits | - low flame luminosity |
| - higher flame speeds | - lower vapor pressure |
| - higher heats of vaporization | - miscibility with water |
| - low cetane number | - toxicity to ecosystems |

Source : modified from [24].

fossil fuels. A dramatic increase in ethanol production using the current cornstarch-based technology may not be practical because corn production for ethanol will compete for the limited agricultural land needed for food and feed production [24]. The lignocellulosic materials such as crop residues, grasses, sawdust, wood chips, municipal and animal solid waste as well as wastepaper shows a potential source for low-cost bioethanol.

## 1. Lignocellulosic material composition

The chemical composition of lignocellulosic compound differs considerably and is influenced by genetic and environmental factors (Table 2). However, cellulose, hemicelluloses and lignin are comprised primarily of lignocellulosic materials. As this table indicates, cellulose account for roughly 50 – 70% of the dry mass of most biomass species, with hemicelluloses and lignin accounting for approximately 30 - 50 and 10 - 30%, respectively as well as ash and minor components (e.g. waxes and protein) make up balance.

Cellulose is a linear polymer of glucose unit which condensed through $\alpha$-1,4-linkages and is usually arranged in microcrystalline structures, which is very difficult to dissolve or hydrolyze under natural conditions. The degree of polymerization (DP) of cellulose chains range from 500 to 25,000 [28, 29]. Unlike, Hemicellulose is a heteropolysaccharide composed of different sugar in addition to glucose especially xylose but also including mannose, galactose, rhamose and arabinose. Hemicellulose is more soluble than cellulose and frequently branched with DP of 100 to 200 [28]. Xylan is the most common hemicelluloses component of grass and wood. Lignin is a highly irregular and insoluble polymer consisting of phenylpropanoid subunits, namely $\rho$-hydroxyphenyl (H-type), guaiacyl (G-type) and syringyl (S-type) units. Lignin has no repeating subunits, thereby making the enzymatic hydrolysis of this polymer extremely difficult.

## 2. The production of bioethanol from lignocellulosic materials

### 2.1 Bioprocess of bioethanol from lignocellulosic materials

A variety of processing schemes can be used to convert lignocellulosic materials to bioethanol. Economic reason is the most important factor. A high conversion yield must be achieved on cellulosic and hemicellulosic sugars [27]. Processing options are largely differentiated by the methods chosen for hydrolyzing cellulose and hemicelluloses to their component sugars (i.e., glucose, xylose, arabinose, galactose and mannose). In particular, cellulose hydrolysis can either precede fermentation or be carried out simultaneously with fermentation. Processing schemes in which cellulose is hydrolyzed to glucose using either cellulase enzymes or concentrated acids at relatively low temperatures (lower than $100^{O}$C) typically degrade less carbohydrate than schemes based on higher temperature acid hydrolysis of cellulose. Enzyme hydrolysis processes are generally considered to offer the best potential for maximizing overall process yields [27]. However, several factors are limited a lignocellulosic to ethanol process such as (i) efficient de-polymerization of cellulose and hemicelluloses to soluble sugars (ii) efficient fermentation of a mixed-sugar hydrolyzate containing six-carbon (hexose) and five-carbon (pentose) sugars as well as fermentation inhibitory compounds (iii) advanced process integration to minimize process energy demand and (iv) cost-efficient use of lignin [21, 24]. Processing of lignocellulosics to bioethanol consists of four major unit operations: size reduction

**Table 2** The chemical composition of common lignocellulosic materials and wastes.

| Lignocellulosic materials | Cellulose (%) | Hemicellulose (%) | Lignin (%) | Etc. (%) |
|---|---|---|---|---|
| **Hardwood species** | 40 – 55 | 24 – 40 | 18 – 25 | - |
| - Silver maple | 47.1 | 21.7 | 20.8 | 10.4 |
| - Sycamore | 44.9 | 20.5 | 22.8 | 11.8 |
| - Black locust | 50.4 | 20.4 | 21.5 | 7.7 |
| - Poplar hybrid NE388 | 49.4 | 17.1 | 21.8 | 11.7 |
| - Poplar hybrid N11 | 52.8 | 13.5 | 22.5 | 11.2 |
| - Sweetgum | 50.2 | 20.2 | 21.8 | 7.8 |
| **Herbaceous species** | 25 – 40 | 25 – 50 | 10 – 30 | - |
| - Switchgrass | 37.8 | 19.4 | 21.9 | 20.9 |
| - Weeping lovegrass | 38.4 | 21.3 | 21.2 | 19.1 |
| - *Sericea lespedeza* | 32.4 | 17.4 | 31.6 | 18.6 |
| - Reed canary grass | 26.1 | 13.1 | 15.6 | 45.2 |
| - Flatpea hay | 30.5 | 10.8 | 24.5 | 34.2 |
| - Coastal Bermuda grass | 25 | 35.7 | 6.4 | - |
| **Softwood species** | 45 – 50 | 25 – 35 | 25 – 35 | - |
| **Agricultural residues** | 40 - 45 | 25 - 35 | 20 - 35 | 8.1 |
| - Corn cobs | 40.5 | 33.9 | 17.5 | 16.2 |
| - Corn stover | 41.9 | 25.2 | 16.7 | - |
| - Nut shells | 25 – 30 | 25 – 30 | 30 – 40 | - |
| - Wheat straw | 30 | 50 | 15 | - |
| - Sorted refuse | 60 | 20 | 20 | - |
| - Leaves | 15 – 20 | 80 – 85 | 0 | - |
| - Cotton seed hairs | 80 - 95 | 5 - 20 | 0 | - |
| **Wastes** | | | | |
| - Newspaper | 40 – 55 | 25 – 40 | 18 – 30 | - |
| - Waste paper from chemical pulps | 60 – 70 | 10 – 20 | 5 – 10 | - |
| - Primary wastewater solids | 8 – 15 | NA[a] | 24 – 29 | - |
| - Swine waste | 6 | 28 | NA[a] | - |

Source : Modified from [19, 27].

NA[a] – not available

and pre-treatment, hydrolysis, fermentation and product separation/distillation. Schematic flowsheet for the bioconversion of biomass to bioethanol is shown in Fig. 1.

### 2.1.1 Size reduction and pre-treatment

Size reduction and pre-treatment is the first step in bioconversion of lignocellulosic materials to bioethanol. The goal of pretreatment technology is to alter or remove structural and compositional impediments to hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicelluloses [30]. The pre-treatment stage promotes the physical disruption of the lignocellulosic matrix in order to facilitate acid or enzyme-catalyzed hydrolysis. Pre-treatments can have significant implications on the configuration and efficiency of the rest of the process and ultimately, also the economics [31]. To assess the cost and performance of pre-treatment technologies, techno-economic analyses have been performed recently [32]. Results have shown that pre-treatment is the most significant determinant of success of the cellulosic bioethanol technology because it defines the extent to and cost at which the carbohydrates of cellulose and hemicelluloses can be converted to bioethanol. A successful pre-treatment must meet the following requirement such as improve formation of sugars or the ability to subsequently from sugars by hydrolysis, avoid degradation or loss of carbohydrate, avoid formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes as well as the process must be cost effective [24].

Pre-treatment can be carried out in different ways such as mechanical pre-treatment, stream explosion, ammonia fiber explosion, supercritical $CO_2$ treatment, alkali or acid pre-treatment, ozone pre-treatment and biological pre-treatment. Comparison of various pre-treatment options is given in Table 3.

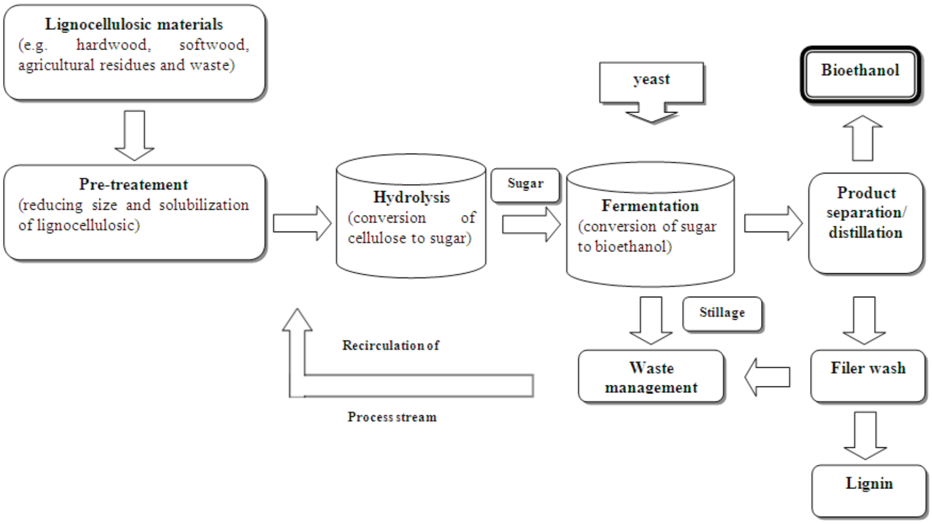

**Fig. 1** Schematic diagram for the bioconversion of lignocellulosic materials to bioethanol
Source : modified from [27].

**Table 3** Comparison of various pre-treatment methods

| Pre-tretment method | Chemicals/ Materials/ Microorganism | Incubation condition | Sugar yield (%) | Advantage | Disadvantage |
|---|---|---|---|---|---|
| **Physio-chemical**<br>- Steam explosion (autohydrolysis) | - steam<br>- steam with the addition of acid catalyst ($H_2SO_4$ and $SO_2$) | 160 – 220$^{O}$C for 1 – 5 min | <65 | - increase crystallinity of cellulose<br>- hemicelluloses is easily hydrolyzed<br>- low energy requirement<br>- no recycling or environmental cost | - low sugar yield<br>- less effective for softwood |
| - Ammonia fiber/ freeze explosion | - liquid ammonia and steam | 12 atm, 90$^{O}$C for 30 min | NA[a] | - simple operation<br>- short process time<br>- effective for corn stover | - need to remove ammonia after pre-treatment<br>- less effective in sugar cane bagasse |
| - Acid pre-treatment | - acid (e.g. $H_2SO_4$, HCl, $C_2H_4O_3$, NO and $H_3PO_4$) | 160$^{O}$C for 2 – 10 min | 75 – 90 | - inexpensive<br>- high sugar yield<br>- well known method and widely used | - less effective for commercial development |
| - Ozonolysis | - ozone | room temperature | 57 | - effectively remove lignin<br>- no toxic residue for downstream process | - expensive<br>- large amount of ozone is required |
| **Physio-chemical**<br>- Alkali pre-treatment | - base [NaOH, $Ca(OH)_2$] | low temperature | 60 – 75 | - effectively remove lignin without having effects on other components | - alkali is converted to irrecoverable salts during pre-treatment<br>- long process time (12 – 48 h) |
| - $CO_2$ explosion | - $CO_2$ | 5.62 MPa | 75 | - effective<br>- no inhibitory compound during process | - $CO_2$ gas presented a suffocation hazard to human<br>- $CO_2$ is a greenhouse gas<br>- fixed $CO_2$ system require a large storage |

**Table 3** (Cont.)

| **Pre-tretment method** | **Chemicals/ Materials/ Microorganism** | **Incubation condition** | **Sugar yield (%)** | **Advantage** | **Disadvantage** |
|---|---|---|---|---|---|
| - Organosolv process | - organic solvent + HCl or $H_2SO_4$ | >185$^{O}$C | NA$^{a}$ | - high yield of xylose can be obtained | - expensive<br>- need to remove all solvents after pre-treatment |
| - Oxidative delignification | - peroxidase enzyme + $H_2O_2$ | 30$^{O}$C for 8 h | 85 | - high sugar yield | - expensive<br>- produced toxic residue |
| **Physical** | | | | | |
| - Pyrolysis | - vacuum pyrolysis<br>- flash vacuum thermolysis | > 300$^{O}$C | ND$^{b}$ | - effective (pyrolysis + acid hydrolysis yield 80 – 85% of sugar) | - high energy consume<br>- complex technology and difficult to operate<br>- high energy consume |
| - Mechanical comminution | - knife mill<br>- hammer mill<br>- mortar | NA$^{a}$ | ND$^{b}$ | - effective in spruce and aspen chips | |
| **Biological treatment** | - white rot fungi | biological degradation | 35 - 77 | - simple<br>- environmental friendly | - long process time<br>- low sugar yield |

NA$^{a}$ – not available

ND$^{b}$ – not detection

Source : modified from [1, 2, 8, 9, 15, 16, 22, 38, 40]

### 2.1.2 Hydrolysis of lignocellulosic materials

After the pre-treatment process, the cellulose is prepared for hydrolysis, meaning cellulose should be degraded into glucose (saccharification) using dilute acid, concentrated acid or enzyme (cellulase) [24]. Hydrolysis without pre-treatment yields typically <20%, whereas yields after pre-treatment often exceed 90%.

A number of processes for hydrolyzing cellulose into glucose have been developed over the year. The vast majority of processing schemes utilize either cellulolytic enzymes or sulfuric acid of varying concentrations. However, lignocellulosic biomass may be hydrolyzed by gamma-ray or electron-beam irradiation or microwave irradiation. Hydrolysis of lignocellulosic biomass is more complicated than that of pure cellulose due to the presence of nonglucan components such as lignin and hemicelluloses [33].

In the former studies, enzymes have been too expensive for economical production of fuel ethanol from biomass. Sulfuric acid, itself, is less expensive than cellulolytic enzymes, although disposal costs associated with the use of sulfuric acid significantly increase its cost.

However, the single largest drawback to using sulfuric acid is that it also readily degrades glucose at the high temperatures required for cellulose hydrolysis [34].

Dilution acid hydrolysis ($H_2S_O4$ and HCl) is the oldest technology for converting cellulose biomass to bioethanol. The process employed under temperatures of 200 – 240$^{O}$C at 1.5% acid concentrations. However, the degradation products from acid hydrolysis are inhibitory to microorganisms. The inhibitory effect of different compounds like furfural, 5-hydroxymethyl furfural (HMF), acetate, hydroxybenzaldehyde (HBA), siringaldehyde (SGA) and vanillin on yeast growth [35]. Two-stage acid hydrolysisis occurred to take advantage of differences between hemicelluloses and cellulose, a first stage is operated under mild conditions (190$^{O}$C, 0.7% acid, 3 min) is recover 5-carbon sugars (e.g. pentoses), while in the second stage, the remaining solids undergoes harsher conditions (215$^{O}$C, 0.4% acid, min) to recover 6 carbon-sugars (e.g. hexose). In this way, 50% glucose yield is obtained [36]. Concentrated acid process using (30 – 70%) $H_2S_O4$ has higher glucose yield (90%) and is relatively rapid (10 – 12 h) but the amount of used acid is a critical economic factor [37].

However, cellulose hydrolysis is currently carried out using microbial cellulolytic enzymes. Both bacteria and fungi can produce cellulase for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Bacteriodes, Erwinia, Acetovibrio, Microbispora and Streptomyces* can produce cellulase [24]. The widely accepted mechanism for enzymatic cellulose hydrolysis involves synergistic actions by endoglucanases or endo-1, 4-β-glucanases (EG), exoglucanases or cellobiohydrolases (CBH) and β-glucosidase (BGL). EG play an important role in the cellulose hydrolysis by cleaving cellulose chains randomly and thus encouraging strong degradation. EG hydrolyze accessible intramolecular β-1,4-glucosidic bonds of cellulose chains randomly to produce new chain ends; exoglucanases processively cleave cellulose chains at the end to release soluble cellobiose or glucose and BGL hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition. BGL complete the hydrolysis process by catalyzing the hydrolysis of cellobiose to glucose [24]. Enzymatic hydrolysis has demonstrated better results for subsequent fermentation because no degradation components of glucose are formed as well as utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis. Enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 35 – 50$^{O}$C) and does not have a corrosion problem [24]. In addition, enzymatic hydrolysis produces better yields than acid catalyzed hydrolysis and enzyme manufacturers have recently reduced cost substantially using modern biotechnology ([38]. However, the hydrolysis process is slow. Due to the fact that structural parameters of substrate, such as lignin and hemicellulose content, surface area and cellulose cystallinity is obstructed.

Enzymatic hydrolysis of recycled paper sludge using different conditions (temperature, residence time, substrate and enzyme loading) were evaluated [39]. The data showed that only Celluclast$^{®}$ 1.5L (commercial name of cellulolytic enzyme) under temperature at 50$^{O}$C pH 5.5 with high enzyme dosage (FPase) dosage of 120 Ug-1 carbohydrate, gave the degree of saccharification (DS) at 45% after 6 days of incubation. However, the highest DS of 100% after 72 h was obtained under temperature of 35$^{O}$C pH 5.5 with Celluclast$^{®}$ 1.5L applied on an FPase dosage of 25 Ug$^{-1}$ carbohydrate.

Under above optimal condition, the supplemented of an excess Novozyme® 188, a cellubiase (β-glucosidase) was not necessary when the hydrolysis gave the same % of DS (100%), meaning that all the cellulose present in the substrate was completely hydrolyzed to glucose [39].

The bioconversion tendencies of various wastepaper materials, a major component of solid waste including newspaper, foolscap paper, office paper, filter paper and cardboard were treated with the cellulase enzyme from *Trichoderma viride.* The result indicated under enzyme concentration of 0.17 mg/ml that foolscap paper and office paper showed maximum bioconversion at an amount of 10 mg paper while filter paper and cardboard were maximally bioconverted at 20 mg and newspaper at 7.5 mg. Under optimal condition wastepaper materials produced sugar concentration of 0.50 – 1.64 μg/ml [4].

### 2.1.3 Fermentation

The classic configuration employed for fermenting biomass hydrolyzates involves a sequential process where the hydrolysis of cellulose and the fermentation are carried out in different units. This configuration is known as separate hydrolysis and fermentation (SHF). In the alternative variant, the simultaneous saccharification and fermentation (SSF), the hydrolysis and fermentation are performed in a single unit. The most employed microorganism for fermentation lignocellulosic hydrolyzated is *Saccharomyces cerevisiae*, which ferments the hexose contained in the hydrolyzate but not the pentoses [37].

Enzymatic hydrolysis performed separately from fermentation step is known as SHF. In the SHF configuration, the joint liquid flow from both hydrolysis reactors first enters the glucose fermentation reactor. The mixture is then distilled to remove the bioethanol leaving the unconverted xylose behind. In the second reactor, xylose is fermented to bioethanol and the bioethanol is again distilled. One of the main features of the SHF process is that each step can be performed at its optimal operating conditions. However, the disadvantage is that cellulolytic enzymes are end-product inhibited so that the rate of hydrolysis is progressively reduced when glucose and cellobiose accumulate [21]. In contrast with SSF, the sugars from pre-treatment and enzymatic hydrolysis steps are fermented in a combined reactor. The SSF process show more attractive indexes than the SHF as higher ethanol yields and less energetic consumption. In this case, the cellulase and microorganisms are added to the same process unit allowing that the glucose formed during the enzymatic hydrolysis of cellulose be immediately consumed by the microbial cells converting it into ethanol [37]. Thus, the inhibition effect caused by the sugars over the cellulase is neutralized. However, the need of employing more dilute media to reach suitable rheological properties makes that final product concentration be low. In addition, this process operates at non-optimal conditions for hydrolysis and requires higher enzyme dosage, which positively influences on substrate conversion, but negatively on process costs. In summary, the production of bioethanol using SHF and SSF is summarized in Table 4.

Recently, the SSF technology has proved advantageous for the simultaneous fermentation of hexose and pentose which is so called simultaneous saccharification and co-fermentation (SSCF). In SSCF, the enzymatic hydrolysis continuously releases hexose sugars, which increases the rate of glycolysis such that the pentose sugars are fermented faster and with higher yield [21]. SSF and SSCF are preferred since both unit operations can be done in the same tank, resulting in lower costs [30].

**Table 4** The biotechnological production of bioethanol from separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF).

| **Substrates** | **Organisms** | **Fermentation /condition** | **Pre - treatment method** | **Enzyme hydrolysis** | **Ethanol concentration (g/l)** | **References** |
|---|---|---|---|---|---|---|
| Rice straw | *S. cerevisiae* | SSF/aerobic | Dilute-acid | Cellulase (15 FPU/g) | 6.83 | [40] |
| | | SSF/anaerobic | | | 10.20 | |
| | *Mucor indicus* | SSF/ aerobic | | | 7.79 | |
| | | SSF/ aerobic | | | 11.35 | |
| Wheat straw | Recombinant *Escherichia coil* | SHF | Alkaline $H_2O_2$ | Celulase + xylanase + β-glucosidase | 18.90 | [41] |
| *Antigonum leptopus* leaves | *S. cerevisiae* | SSF/ aerobic | Alkaline $H_2O_2$ | Cellulase (40 FPU/g) | 19.00 | [42] |
| Sugar cane leaves | | | | | 18.00 | |
| Solka floc | | | | | 23.00 | |
| *A. leptopus* leaves | *kluyveromyces fragils* | SSF/ aerobic | Alkaline $H_2O_2$ | Cellulase (40 FPU/g) | 23.00 | [42] |
| Sugar cane leaves | | | | | 25.00 | |
| Solka floc | | | | | 30 | |
| *A. leptopus* leaves | *S. cerevisiae* | SSF/ aerobic | Alkaline $H_2O_2$ | Celulase (40 FPU/g) + β-glucosidase (50 IU/g) | 21.00 | [42] |
| Sugar cane leaves | | | | | 22.00 | |
| Solka floc | | | | | 25.00 | |
| *A. leptopus* leaves | *K. fragilis* | SSF/ aerobic | Alkaline $H_2O_2$ | Celulase (40 FPU/g) + β-glucosidase (50 IU/g) | 27.00 | [42] |
| Sugar cane leaves | | | | | 28.00 | |
| Solka floc | | | | | 35.00 | |
| Recycled paper sludge | *Pichia stipitis* CBS 5773 | SHF/ aerobic | Alkaline $H_2O_2$ | Celulase (25 FPU/g) + β-glucosidase | 19.47 | [39] |
| | | SSF/ aerobic | | | 18.72 | |
| Yeast malt extract (YMP) - control | | SSF/ aerobic | | | 26.60 | |

Table 4 (cont.)

| **Substrates** | **Organisms** | **Fermentation / condition** | **Pre-treatment method** | **Enzyme hydrolysis** | **Ethanol concentration (g/l)** | **References** |
|---|---|---|---|---|---|---|
| High-solid paper sludge | *S. cerevisiae* | SSF/ aerobic | Ball milling + Phosphoric acid | Celulase (20 FPU/g) | 30.48 | [1] |
| Wastewater | *S. cerevisiae* | SSF/ aerobic | - | Celulase | 2.30 | [43] |
| Cotton-based waste textile | *S. cerevisiae* CCUG 53310 | SSF/ aerobic | Phosphoric acid | Celulase (60 FPU/g) + | 14.00 – 14.50 | [44] |
|  |  |  | Alkaline NaOH | β-glucosidase (5.2 IU/mg) | 48.00 |  |

### 2.1.4 Product separation/distillation

As biomass hydrolysis and fermentation technologies approach commercial viability, advancements in product recovery technologies will be required. Distillation is often the technology of choice, in which fermentation products are more volatile than water. Distillation technologies that will allow the economic recovery of dilute volatile products from streams containing a variety of impurities have been developed and commercial demonstrated [45]. A distillation system separates the bioethanol from water in the liquid mixture. Water content of virgin bioethanol is generally higher than 80%. Large quantities of energy are required to concentrate the ethanol to 95.6% (azeotrope mixture of ethanol with water). The beer column separates most of the bioethanol from water (and solids, if any) and produces a top stream rich in bioethanol and a bottom stream rich in water [24]. In this flow, bioethanol from cellulosic biomass has likely lower product concentrations (≤ 5 wt%) than in bioethanol from corn. The maximum concentration of bioethanol tolerated by the microorganisms is about 10 wt% at 30OC but decreases with increasing temperature. To maximize cellulase activity, the operation is rather at maximum temperature (37$^{O}$C), since the cost impact of cellulase production is high relative to distillation [24].

The first step is to recover the bioethanol in a distillation or beer column, where most of the water remains with the solids part. The product (37 % bioethanol) is then concentrated in a rectifying column to a concentration just below the azeotrope (95 %) [36]. The remaining bottom product is fed to the stripping column to remove additional water, with the bioethanol distillate from stripping being recombined with the feed to the rectifier [46]. The recovery of bioethanol on the distillation columns in the plant is fixed to be 99.6 % to reduce bioethanol losses [24].

After the first effect, solids are separated using a centrifuge and dried in a rotary dryer. A portion (25%) of the centrifuge effluent is recycled to fermentation and the rest is sent to the second and third evaporator effects. Most of the evaporator condensate is returned to the process as fairly clean condensate (a small portion, 10 %, is split off to waste water treatment to prevent build-up of low-boiling compounds) and the concentrated syrup contains 15-20 % by weight total solids [24].

## 3. Bioethanol status

Total annual world production of non-beverage ethanol is estimated to be 8 – 20 billion liters, with most of the production occurring in the United State of America followed by Europe [27, 37]. In the United State, the use of ethanol as an oxygenated/fuel additive in 90 % gasoline – 10 % ethanol blends has increased significantly during the past 15 years. The estimated cost of production ethanol from cellulosic materials varies widely between investigations with production costs in range of 25 – 70 US$/barrel ethanol and in more recent techno-economic evaluation [47]. The costs of producing bioethanol in Brazil are the world's lowest (25 – 30 US$ a barrel). Other efficient sugar producing countries such as Pakistan, Swaziland and Zimbabwe have production costs similar to Brazil [24]. Most cost estimations are based on laboratory-scale and to some extent, pilot scale data for individual process steps and should be treated with caution. The cost of raw material, which varies considerably between different studies (US$22 – US$61 per metric ton dry matter) and the capital cost, which makes the total cost dependent on plant capacity, contribute most to the total production cost. The cost for hydrolysis, particularly for the enzymatic process, is also a major cost contributor [21].

Raw material cost is reduced by selecting of the most appropriate feedstock for ethanol production strongly depends in the local conditions. As well as ideal materials for ethanol production was required for a complete hydrolysis of both cellulose and hemicelluloses with a minimum of sugar degradation, followed by efficient fermentation of all sugars in the biomass [37]. Lignocellulosic materials represent a promising option as a feedstock for ethanol production considering their output/input energy ratio, their great availability both in tropical and temperature countries, their low cost (primarily related to their transport) and their ethanol yields. One of the advantages of the use of lignocellulosic biomass is that this feedstock is not directly related to food production. This implies the production of bioethanol without the need of employing vast extensions of fertile cultivable land for cropping cane or corn exclusively dedicated to the bioenergy production. In addition, lignocellulosic is a resource that can be processed in different ways for production of many other products like synthesis gas, methanol, hydrogen and electricity [48]. However, the production of bioethanol from cellulosic materials is combined with integration of energy-intensive process steps (e.g. pretreatment, distillation, evaporation and drying). Future reductions in the energy demand can be obtained by recycling certain process streams, to minimize the amount of fresh water used. Process integration reduces the capital costs. More recently, the SSCF technology has proved advantageous for hydrolysis and fermentation in a single reactor, using one or a mixture of microorganisms that produce all the required enzymes and ferment all sugars- so called consolidated bioprocessing (CBP). However, no such microorganisms are currently available and the concept is subject to future research [49].

Currently, the Iogen Corp. demo-plant is the only operating plant for the production of bioethanol from lignocellulosic material using enzymatic hydrolysis process. The plant can handle up to 40 tonnes per day of wheat, oat and barley straw and is designed to produce up to 3 million liters of cellulose ethanol per year. Abengoa Bioenergy is also constructing a pilot plant in New York, USA, to convert residual starch, cellulose and hemicelluloses (corn stover) to bioethanol and high-protein feed. In Salamanca, the same company constructed a demonstration plant integrated with a fuel ethanol from grain plant, producing 195 million liters. In the demonstration plant, an additional 5 million liters of ethanol per year will be produced from cellulose mainly from wheat staw. In Sweden, a fully integrated pilot plant for ethanol production from softwood, comprising both two-stage dilute acid hydrolysis and the enzymatic process, was taken into operation in mid 2004. The pilot has a maximum capacity of 2 ton wood per day [21].

## 4. Conclusion and future direction

Energy consumption has increased steadily over the last century. Crude oil has been the major resource to meet the increased energy demand. However, the decline in worldwide crude oil production will begin before 2010. As well as the annual global oil production would decline from the current 25 billion barrels to approximately 5 billion barrels in 2050. Ethanol is the most employed liquid biofuel either as a fuel or a gasoline enhancer [19]. Many researchers reported on the production of ethanol from various raw materials. The fuel ethanol obtained from crops and lignocellulosic biomass showed the great result. One of the advantages of bioconversion with lignocellulosic is the opportunity to create a biorefinery, producing value-added co-products plus fuel bioethanol. For instance, sugars may be subjected to bacterial fermentation under aerobic and anaerobic conditions, producing a variety of other products including lactic acid, which in turn may be processed into plastics and other products. The non-carbohydrate components of lignin also have potential for use in value-added application [24, 31]. The future research on ethanol production has been explored using bacteria. Moreover, the improvement of enzymatic hydrolysis is also investigated. The potential for the production of bioethanol seems to be limited by the production cost. Therefore, the chance to obtain cheap bioethanol will depend on the successfully improving the enzymatic hydrolysis with efficient enzymes, reduce enzyme production cost and novel technology for high solids handling. Furthermore, development of fermenting organisms which are more tolerant to inhibitors and ferment all sugars in the raw material in concentrated hydrolyzates at high productivity and with high ethanol concentration. As well as extending process integration to reduce the number of process step and the energy demand and to re-use process streams to eliminate the use of fresh water and to reduce the amount of waste streams [21]. Even so this method appears to be a realistic goal for the future.

## References

[1] Yamashita, Y., Sasaki, C. and Nakamura, Y. (2010). Development of efficient system for ethanol production from paper sludge pretreated by ball milling and phosphoric acid. **Carbohydrate Polymers. 79,** 250 – 254.

[2] Araque, E., Parra, C., Freer, J., Contreras, D., Rodriguez, J. Mendonca, R. and Baeza, J. (2008). Evaluation of organosolv pretreatment for the conversionof *Pinus radiata* D. Don to ethanol. **Enzyme and Microbial Technology. 43,** 214–219.

[3] Ohgren, K., Rudolf, A., Galbe, M. and Zacchi, G. (2006). Fuel ethanolproduction from steam-pretreatedcorn stover using SSF at higher dry matter content, **Biomass and Bioenergy. 30,** 863–869.

[4] van Wyk, J.P.H. and Mohulatsi, M. (2003). Biodegradation of wastepaper by cellulase from *Trichoderma viride*. **Bioresource Technology. 86,** 21 – 23.

[5] Yu, P. (1996). Analysis of municipal recyclable materials recycling program. **Resource, Conservation and Recycling. 17,** 47 – 56.

[6] Wyman, C.E. and Goodman, B.J. (1993). Biotechnology for production of fuels, chemicals and materials from biomass. **Applied in Biochemistry and Biotechnology. 39,** 39 – 59.

[7] Hocking, M.B. (1991). Paper versus polystyrene: A complex choice. **Science. 251,** 504 – 505.

[8] van Wyk, J.P.H. (1999). Hydrolysis of pretreated paper materials by different concentration of cellulase from *Penicillium funiculosum*. **Bioresource Technology. 69,** 269 – 273.

[9] van Wyk, J.P.H. and Botha, A.C. (1997). Hydrolysis of cellulose materials during successive treatment with cellulase from *Penicillium funiculosum*. **Biotechnolgy Letter. 19,** 687 – 689.

[10] Nazhad, M.M., Ramos, L.P., Paszner, L. and Saddler, J.N. (1994). Structural constraints affecting the initial enzymatic hydrolysis of recycled paper. **Enzyme Microbiology and Technology. 17,** 74 – 86.

[11] Gregg, D.J. and Saddler, J.N. (1996). Factors affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process. **Biotechnology and Bioengineering. 51,** 375 – 383.

[12] Grethelin, H.E. (1985). The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulosic substrates. **Biotechnology. 3,** 155-160.

[13] van Soest, P.J. (1994). **The nutritional ecology of the ruminant, 2nd** ed. Cornell University Press. Ithaca, NY.

[14] Fahey, G.C., Bourguin, L.D., Titgemeyer, E.C. and Atwell, D.G. (1992). **Post-harvest treatment of fibrous feedstuffs to improve their nutritive value.** In: Hung, H.G., Buxton, D.R., Hatfield, R.D. and Ralph, J. (Eds.), Forage Wall Structure and Digestibility. American Society of Agronomy, Madison, WI.

[15] Sawada, T., Nakamura, Y., Kobayashi, F., Kuwahara, M. amd Watanabe, T. (1995). Effects of fungal pretreatment and steam explosion pretreatment on enzymatic saccharification of plant biomass. **Biotechnology and Bioengineering. 48,** 719 – 724.

[16] Gossett, J.M., Healy Jr., J.B., Owen, W.F., Stuckey, D.C., Young, L.Y. and P.L. McCarty. (1976). **Heat treatment of refuse for increasing anaerobic biodegradability.** Final Report. ERDA/NST/7940-7612. National Technical Invormation Service, Springfield, VA.

[17] Lee, J.H., Brown, R.M., Jr., Kuga, S., Shoda, S., and Kobayashi, S. (1994). Assembly of synthetic cellulose **I. Proceeding of the National Academy of Science. 91,** 7425-7429.

[18] Foyle, T., Jenning, L. Mulcahy, P. (2007). Compositional analysis of lignocellulosic materials: evaluation of methods used for sugar analysis of waste paper and straw. **Bioresource Technology. 98,** 3026 – 3036.

[19] Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production : a review. **Bioresource Technology.83,** 1 – 11.

[20] Campbell, C.J. and Laherrere, J.H. (1998). The end of cheap oil. **Scientific American. 3,** 78 -83.

[21] Hahn-Hägerdal, B., Galbe, M., Gorwa-Grauslund, M.F., Lidén, G. and Zacchi, G. (2006). Bio-ethanol– the fuel of tomorrow from the residues of today. **TRENDS in Biotechnology. 24,** 549 – 556.

[22] Yu, Z. and Zhang, H. (2003). Pretreatments of cellulose pyrolysate for ethanol production by *Saccharomyces cerevisiae, Pichia* sp. YZ-1 and *Zymonas mobilis.* **Biomass and Bioenergy. 24,** 257 – 262.

[23] Dermirbas, F., Bozbas, K. and Balat, M. (2004). Carbon dioxide emission trends and environmental problems in Turkey. **Energy Explor Exploit. 22,** 355 – 365.

[24] Balat, M., Balat, H. and öz, C. (2008). Progress in bioethanol processing. **Progress in Energy and Combustion Science. 34,** 551 – 573.

[25] Kim, H., Choi, B., Park, S. and Kim, Y.K. (2005). **Engine performance and emission characteristics of CRDI diesel engine equipped with the WCC and the DOC. Using ethanol blended diesel fuel.** In: Proceeding of the 15th international symposia on alcohol fuels (ISAF XV), San Diego, The United State.

[26] Kadiman, O.K. (2005). **Crops: beyond foods.** In: Proceeding of the 1st international conference of crop security, Malang, Indonesia.

[27] McMillan, J.D. (1997). Bioethanol production : status and prospects. **Renewable Energy. 10,** 295 – 302.

[28] Kuhad, R.C., Singh, A., Eriksson, K.E.L. (1997). **Microorganisms and enzymes involved in the degradation of plant fibre cell walls.** In: Eriksson, K.E.L. (ed.). Advances in Biochemical Engineering/Biotechnology. Springer-Verlag, Germany, pp. 46-125.

[29] Leschine, S. B. 1995. Cellulose degradation in anaerobic environments. **Annual Review of Microbiology. 49,** 399-426.

[30] Mosier, N., Wyman, C., Dale, B., Elander, R., Holtzapple, Y.Y.L.M. and Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. **Bioresource Technology. 96,** 673 – 686.

[31] Mabee, W.E., Gregg, D.J., Arato, C., Berlin, A., Bura, R., Gilkes, N., Mirochnik, O., Pan, X., Pye, E.K. and Saddler, J.N. (2006). Updates on softwood-to-ethanol process development. **Applied in Biochemistry and Biotechnology. 129 - 132,** 55 – 70.

[32] Chen, Y., Sharma-Shivappa, R.R. and Chen, C. (2007). Ensiling agricultural residues for bioethanol production. Applied in **Biochemistry and Biotechnology. 143,** 80 – 92.

[33] Zhang, Y.H.P. and Lynd, L.R. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. **Biotechnology and Bioengineering. 88,** 797 – 824.

[34] Mosier, N.S., Ladisch, C.M. and Ladisch, M.R. (2002). Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. **Biotechnology and Bioengineering. 79,** 610–618.

[35] Rao, R.S., Jyothi, C.P., Prakasham, R.S., Sarma, P.N. and Rao, L.V. Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis.* **Bioresource Technology. 97,** 1974 – 1978.

[36] Hamelinck, C.N., van Hooijdonk, G. and Faaij, A.P.C. (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. **Biomass Bioenergy. 28,** 384 – 410.

[37] Sánchez, O.J. and Cardona, C.A. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. **Bioresource Technology. 99,** 5270 – 5295.

[38] Pan, X., Arato, C., Gilkes, N., Gregg, D., Mabee, W., Pye, K.L., Xiao, Z., Zhang, X. and Saddler, J. (2005). Biorefining of softwoods using ethanol organosolv pulping: preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-product. **Biotechnology and Bioengineering. 90,** 473 – 4814.

[39] Marques, S., Alves, L., Roseiro, J.C. and G′rio, F.M. (2008). Conversion of recycled paper sludge to ethanol by SHF and SSF using *Pichia stipitis.* **Biomass and Bioenergy. 32,** 400 – 406.

[40] Karimi, K., Emtiazi, G. and Teherzadeh, M.J. (2006). Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with *Mucor indicus, Rhizopus oryzae* and *Saccharomyces cerevisiae.* **Enzyme and Microbial Technology. 40,** 138 – 144.

[41] Saha, B.C. and Cotta, M.A. (2006). Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. **Biotechnology Progress. 22,** 449 – 453.

[42] Hari Krishna, S., Prasanthi, K.,Chowdary, G. and Ayyanna, C. (1998). Simultaneous saccharification and fermentation of pretreated sugar cane leaves to ethanol. **Process Biochemistry. 33,** 825 -830.

[43] Cheung, S.W. and Anderson, B.C. (1997). Laboratory investigation of ethanol production from municipal primary wastewater solids. **Bioresource Technology. 59,** 81 – 96.

[44] Jeihanipour, A. and Taherzadeh, M.J. (2009). Ethanol production from cotton-based waste textiles. **Bioresource Technology. 100,** 1007 – 1010.

[45] Madson, P.W. and Lococo, D.B. (2000). Recovery of volatile products from dilute high-fouling process stream. **Applied in Biochemistry and Biotechnology. 84 - 86,** 1049 – 1061.

[46] Kwiatkowski, J.R., Mcaloon, A.J., Taylor, F. and Johnston, D.B. (2006). Modeling the process and costs of fuel ethanol production by the corn dry-grind process,**Industrial Crop and Products. 23,** 288 – 296.

[47] Aden, A., Ruth, M., Ibsen, K., Jechura, J., Neeves, K., Sheehan, J., Wallace, B., Montaque, L., Slayton, A. and Lukas, J. (2002). **Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover.** Technical Report NREL/TP-510-32438. Golden, CO (USA), National Renewable Energy Laboratory. 143 p.

[48] Chum, H.L. and Overeb, R.P. (2001). Biomass and renewable fuels. Fuel **Processing Technology. 71,** 187 – 195.

[49] Lynd, L.R., van Zyl, W.H., McBride, J.E. and Laser, M. (2005). Consolidated bioprocessing of cellulosic biomass: an update. Current Opinion in **Biotechnology. 16,** 577 – 583.