

Challenges and Opportunities for Producing Bioethanol from Lignocellulosic Biomass

D. Kareemulla, Sudha Tyagi, Jaya Rawat, P.V.C. Rao and N.V. Choudary
Corporate R&D Center, Bharat Petroleum Corporation Limited, Greater Noida (201306), India

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ABSTRACT

Energy is the lifeline of global economy. Diminishing fossil fuel reserves and increased concerns over environmental pollution accelerated the need to look for renewable and environmentally sustainable energy sources. In this context, ethanol derived from biomass is advocated as a prominent contributor to offer a potential means to meet our energy needs. Being abundant and outside the human food chain makes lignocellulosic biomass, suitable and sustainable for ethanol production. Several schemes for the conversion of lignocellulosics into sugars have already been demonstrated in laboratory and pilot plant scale. The general procedure involves pretreatment of feedstock followed by hydrolysis to obtain fermentable sugars which are then converted to ethanol by fermentation. The present paper reviews the technological advancements made to date and the challenges involved in each of these processing steps. A procedure to calculate theoretical ethanol yields is also described.

KEYWORDS

Bioethanol, Biomass

1. INTRODUCTION

Diminishing fossil fuel reserves, ever escalating fuel prices, increased concerns over environmental pollution, and most importantly the quest for energy independence and energy security prompted the global research community to accelerate the need to look for renewable and environmentally sustainable energy sources. In this context, ethanol derived from biomass is advocated as a potential alternative to conventional fossil fuels.

Bio-ethanol is mainly produced by fermentation process, although it can also be manufactured synthetically from petroleum (hydration of ethylene). In 1995, about 93% of ethanol in the world was produced by fermentation while about 7% is by the synthetic method. Ethanol is also commercially produced in the pulp and paper industry as a by-product. Currently Brazil and US are the world leaders in bioethanol production using sugarcane and corn respectively. The world ethanol production in 2004 was estimated to be 40 giga litres of which 60% was accounted by Brazil and US together [1]. In India, ethanol is mainly produced from sugarcane molasses.

Though biomass-to-ethanol industry, around the globe, mostly employs sugar and starchy biomass feed stocks, this option is limited to the cases where actual and sustainable surplus of these crops occur. Moreover, since sugar and starchy feed stocks are a part of human food chain, this correlation between food and ethanol market may generate a volatility of the ethanol prices. In developing countries like India, the possible competition with food is one of the risky factors when using agricultural crops for ethanol production. In this regard, lignocellulosic biomass, which is not a part of human food chain, is envisaged to supply a significant portion of the feed-stocks for bio-ethanol production in the medium and long term due to their low cost and abundance.

2. ETHANOL FROM LIGNOCELLULOSIC BIOMASS

2.1 COMPOSITION OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is a complex mixture of holocellulose (cellulose and hemicellulose) carbohydrate polymers, lignin, and a smaller amount of other compounds generally known as extractives [2]. Cellulose is a linear, crystalline homopolymer, made up of repeated units of glucose strung together with beta-glucosidic bonds. Hemicellulose is a highly branched heteropolymer consisting of xylose, glucose, galactose, mannose, and arabinose. The exact sugar composition of holocellulose varies depending on the plant type and origin as well.

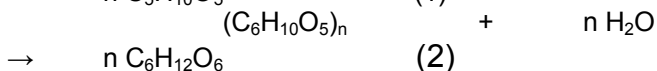
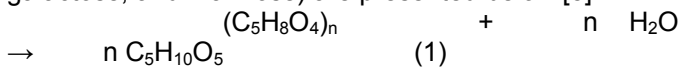
Numerous research efforts have been made on the conversion of lignocellulosic biomass to ethanol over the last two decades. However, none of them are turned out to be cost-effective due to high costs involved and low ethanol yields. Extensive research efforts are underway by industry and academia in making this technology technically and economically viable in near future. The production of ethanol utilizing lignocellulosic biomass feed stocks, generally, consists of four major unit operations: (1) Pretreatment (2) Depolymerization or saccharification of holocellulose fraction (3) Fermentation of mixed free sugars to produce ethanol. (4) Separation and purification. All these unit operations have been widely investigated, and many technological improvements have been proposed so as to enhance the yield of ethanol and to reduce the overall process economics. The process flow diagram is shown in Fig. 1.

2.2 PRETREATMENT

Lignin, which extends structural support for the plant and contains no sugars, encloses the holocellulose carbohydrate polymers making them difficult to break down during the process of saccharification. In order to remove the protecting lignin and to make the carbohydrate fraction accessible in the subsequent saccharification process by acid/enzymatic hydrolysis, the lignocellulosic biomass must be pretreated. Pretreatment affects the structure and chemical composition of the biomass feed stock by extracting lignin (delignification), solubilizing hemicellulose, reducing cellulose crystallinity, and increasing the available surface area and pore volume [3]. A variety of pretreatment technologies have been proposed and explored: physical, physio-chemical, chemical, and biological. Detailed descriptions of each of these pretreatment processes can be found in literature[4]. Thermo-chemical processing options appear more promising than biological options for the delignification of lignocellulosic biomass. However, thermo-chemical pretreatment can have detrimental consequences if the subsequent saccharification is carried out by enzymatic hydrolysis without detoxification. The focus lately has been the development of low-cost reactors and processes such that pretreatment represents a relatively small portion of the total ethanol production costs. Pretreatment of lignocellulosic biomass in an efficient and cost effective manner is a major challenge in producing ethanol from cellulose.

2.3 DEPOLYMERIZATION OR SACCHARIFICATION OF HOLOCELLULOSE

After pretreatment there are two distinct processes to depolymerize holocellulose fraction into monomeric sugars namely: acid hydrolysis and enzymatic hydrolysis. The basic hydrolysis reactions of 5-carbon (xylose and arabinose) and 6-carbon sugars (glucose, galactose, and mannose) are presented below [5].



2.3.1 ACID HYDROLYSIS

Acid-catalyzed hydrolysis has been applied to both cellulose and hemicellulose components of lignocellulosic biomass. Two types of acid hydrolysis processes are commonly used: dilute acid hydrolysis and concentrated acid hydrolysis.

Dilute Acid Hydrolysis

In dilute acid hydrolysis the hemicellulose fraction is depolymerized at lower temperatures than the cellulose fraction. If higher temperatures or longer residence times are applied, the monosaccharides formed from hemicellulose will undergo degradation giving rise to fermentation impediments such as furan compounds, weak carboxylic acids, and phenolic compounds.

Therefore, in order to reduce the degradation of sugars and to improve the efficiency of fermenting step, acid hydrolysis is normally carried out in two stages [6]: in the first stage, biomass is treated with dilute acid at relatively mild conditions during which the hemicellulose fraction gets hydrolyzed yielding xylose and other sugars (glucose, galactose, mannose, and arabinose). The liquid stream containing the monosaccharides is recovered, thereby avoiding degradation and the separated solid material is then sent to second stage wherein it is treated at a higher temperature which results in the depolymerization of more resistant cellulose fraction into glucose. Avoiding degradation is important not only to improve the yield, but also to avoid inhibition problems, since the degradation products are extremely toxic to the fermenting organism. Several chemical and biological methods have been used for the detoxification of acid hydrolysates which include overliming, charcoal adsorption, ion exchange, and enzymatic treatment by laccase. However, the cost involved is often higher than the benefits achieved. A research team at the National Renewable Energy Laboratory (NREL) has significantly advanced the dilute acid hydrolysis technology by exploiting the bed shrinking phenomena to improve the reactor performance. A modeling study has proven that the shrinking-bed operation indeed positively affects hemicellulose hydrolysis [7].

Concentrated Acid Hydrolysis

This process is based on concentrated acid depolymerization of holocellulose followed by a dilution with water to dissolve the substrates into sugar constituents. This process employs relatively mild temperatures and the only pressures involved are those created by pumping materials from vessel to vessel. This process enables complete and rapid conversion of cellulose to glucose and hemicellulose to xylose with a little degradation. The mild temperatures and low pressures used also facilitate the use of low cost materials. The primary advantage of this process is its high sugar recovery efficiency; about 90% of both hemicellulose and cellulose fraction gets depolymerized into their respective monomeric sugars. Acid recovery and acid re-concentration are very crucial in order to make this process technically and economically feasible. Normally the acid and sugar syrup are separated via ion exchange and the acid is reconcentrated using multiple effect evaporators.

2.3.2 ENZYMATIC HYDROLYSIS

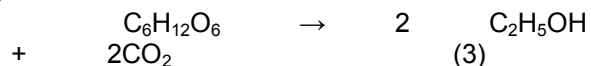
Enzymes are relatively newcomers with respect to biomass-to-ethanol processing. Enzymes are naturally occurring plant proteins that cause the holocellulose chains to break down in order to produce monosaccharides for the subsequent fermentation step. There has been a tremendous interest in the enzymatic hydrolysis of acid, alkaline, and biologically pretreated lignocellulosic biomass as it has demonstrated better results during the fermentation of monosaccharides because no degradation products are formed although

the process is inherently slower. The other prominent advantages of enzymatic hydrolysis are: mild process conditions which do not require expensive materials of construction and low process energy requirements.

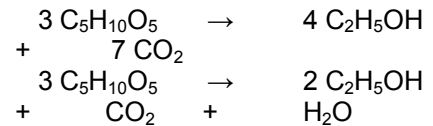
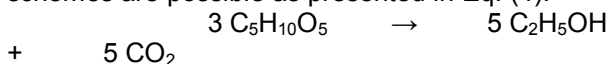
Enzymatic hydrolysis of cellulose and hemicellulose is achieved using cellulases and hemicellulases. Bacteria and fungi are the good sources of cellulases and hemicellulases. The classical cellulase system includes endoglucanase, exoglucanase, and cellobiase. Hemicellulases include enzymes that break down β -1, 4-xylan and various other side chains. Commercial development of hemicellulases for enzymatic hydrolysis of lignocellulosics is not as advanced as cellulases because current commercial preparations have been primarily on dilute-acid pretreated biomass where hemicellulose is removed before saccharification [8]. However, if the pretreatment is non-acidic, where the hemicellulose fraction remains intact, hemicellulases are required. Current cellulases tend to have weak hemicellulose activity and are not adequate for the complete conversion of hemicellulose fraction of lignocellulosics. Development of low-cost, commercial hemicellulases that work synergistically with cellulases is one of the goals of the current research activities. The efficiency of enzymatic hydrolysis is governed by many factors which include: type of pretreatment, composition of enzymes, thermostability of enzymes, their ability to adsorb on the substrate, the site of enzymatic attack within the polymer, type of sugars released, inhibition of enzymatic activity by the end-products of the biodegradation, duration of the hydrolysis, location and structure of lignin, and pH of the medium [9]. Several approaches have been utilized to improve cellulase performance and to decrease the amount of enzyme needed to saccharify biomass substrates. The cost of enzymes is another important factor that contributes to the total cost of fuel ethanol from biomass accounting about 20% [10]. Significant progress has been made in recent years in the cost reduction of cellulases; however, cellulases are still very expensive.

2.4 FERMENTATION

Once the cellulose and hemicellulose fractions are broken down to simple C5 and C6 sugars, fermentation will be then carried out to produce ethanol and other by-products as discussed below [11]. These by-products include carbon dioxide, and water. Fermentation of 1 mole of C6 sugar yields 2 moles of ethanol as shown in Eq. (3).



Fermentation of C5 sugars is not as straight forward as the fermentation of C6 sugars. Depending on the micro-organism and operating conditions, several fermentation schemes are possible as presented in Eq. (4).



Production of ethanol from glucose has been commercially dominated by the yeast *S. cerevisiae* (baker's yeast). However, sugar derived from lignocellulosic biomass is a mixture of hexoses (primarily glucose) and pentoses (primarily xylose), and most wild-type strains of *S. cerevisiae* can not metabolize pentoses. Therefore micro-organism development to ferment the pentoses (arabinose and xylose) with high yields is crucial in making cellulosic ethanol economically viable [12]. The most promising yeasts that have the ability to use both pentoses and hexoses are *Pichia stipitis*, *Candida shehatae*, and *Pachysolan tannophilus*. Of late, Ethanol Producing Bacteria (EPB) has gained much attention, because of their higher growth rate than that of the conventionally used microorganisms for the commercial production of bio-ethanol. The engineered ethanologenic bacteria that currently show the most promise for industrial exploitation are *E.coli*, *K. oxytoca*, *X. mobilis*, *clostridium cellulolyticum* and *Lactobacillus casei*. These EPBs are expected to make industrial ethanol production more economical; however, its narrow spectrum of fermentable carbohydrates is currently hampering its industrial exploitation [13]. To get around this problem, a number of groups have taken on the challenge of developing recombinant organisms that have the ability to ferment both hexoses and pentoses. Further, in contrast to the clean sugar streams derived from starch and sucrose, hydrolysates derived from biomass tend to have fermentation inhibitors that either must be removed when concentrations are too high or require the development of robust strains that are recalcitrant to the inhibitors.

Significant progress has been made in recent years in all aspects of lignocellulosic conversion to ethanol as discussed above. In particular, some process schemes involving reaction-reaction integration are being developed as discussed below. The most important process integration made is the introduction of Simultaneous Saccharification and Fermentation (SSF), which enables one-step process of sugar production and fermentation into ethanol. This approach has been further improved by combining enzymatic hydrolysis of holocellulose with simultaneous co-fermentation of multiple sugar substrates to produce ethanol and is named, Simultaneous Saccharification and Co-Fermentation (SSCF) [14]. In comparison to the process where these two steps (saccharification and fermentation) are sequential, the SSF and SSCF approaches enable higher yields of ethanol by preventing end-product inhibition, as well as by eliminating the need for separate reactors for saccharification and fermentation. Another prominent advantage of these approaches is a shorter processing time which could facilitate continuous processing. In

spite of these advantages presented by SSF, it has, however, some drawbacks. These lie mainly in different temperature optima for saccharification/hydrolysis (45-50°C) and fermentation (28-35°C) and difficulty in recycling the yeast. Thermo tolerant yeast could be more suitable in this case at industrial level.

3. THEORETICAL ETHANOL YIELD CALCULATION

In addition to sustainability, the potential of a particular biomass feedstock in producing ethanol is another important factor to be seriously considered. Theoretical ethanol yield estimation would be useful in assessing the potential of feedstock and also optimizing the process. From the basic hydrolysis reactions presented in section 2.3, 1 kg of any C₆ sugar polymer yields 1.11 kg of respective C₆ sugars while 1 kg of any C₅ sugar polymer yields 1.136 kg of respective C₅ sugars. Similarly, from the basic fermentation reactions presented in section 2.4, fermentation of 1 kg of any C₆ sugar yields 0.51 kg of ethanol while the fermentation of 1 kg of any C₅ sugar polymer yields 0.51-0.20 kg of ethanol depending upon the fermentation schemes as discussed in section 2.4. A sample theoretical ethanol yield calculation for corn Stover is described here. Corn Stover constitutes 40.1 % and 24.0 % 6-carbon and 5-carbon sugars respectively [11]. The rest includes non-ethanol producing constituents.

Theoretical ethanol yield from 6-carbon sugars
 $= 0.401 \times 1.11 \times 0.51 = 0.2270$ kg ethanol/kg
 corn Stover (5)
 Similarly, ethanol yield from 5-carbon sugars
 $= 0.24 \times 1.136 \times 0.51 = 0.1390$ kg ethanol/kg
 corn stover (maximum)
 $= 0.24 \times 1.136 \times 0.20 = 0.0545$ kg ethanol/kg
 corn stover (minimum)

Therefore the total theoretical ethanol yield = (5) + (6)
 $= 0.366 -$
 0.2815 kg ethanol/kg corn stover

4. CONCLUSIONS

Significant progress has been made in recent years in all aspects of lignocellulosic conversion to ethanol; challenges are still there which need to be addressed. Minimum by-products, developing more efficient pretreatment technologies and microorganism that could metabolize a wide range of major sugars are the desired characteristics for the commercial ethanol production. The cost of feed stock and cellulolytic enzymes, which account for about 40% and 20% of ethanol production cost respectively, are two important parameters contributing to the cost of ethanol production. Recent technological advancements significantly reduced the cost of biomass-to-ethanol conversion; however, there is still a huge scope to further bring down the cost by increased research efforts. Data is still needed on material and energy balances to validate the existing integrated pilot-scale results.

ACKNOWLEDGEMENTS:

The authors express their sincere thanks to Dr.M.A.Siddiqui, ED (R&D) BPCL for permitting to present this paper.

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Figure .1 process flow diagram of bio-ethanol from lignocellulosic biomass

Table 1: Theoretical Ethanol yields for different feed stocks

Feed stock	% Hexose	% Pentose	Theoretical ethanol yield (kg ethanol/kg feed stock)
Sugarcane	33	30	0.361 –
bagaase	30	24	0.255
Wheat	33	18	0.309 –
straw	32	24	0.224
Sorghum	41	16	0.291 –
straw	42	39	0.228
Rice straw	35	15	0.320 –
Oat straw	40	20	0.236
Corn cob	38	36	0.325 –
Corn stalks	36	15	0.268
Barley			0.464 –
straw			0.326
Ground nut			0.285 –
shell			0.232
Rice hulls			0.342 –
			0.272
			0.424 –
			0.297
			0.291 –
			0.238

