Approaches of Increasing Efficiency and Cost Reduction in Production of Cellulosic Ethanol

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Abstract- **In a world of rapidly rising carbon emission and growing unease about imported oil, the appeal of renewable fuel is growing apace. The most common renewable fuel today is ethanol produced from sugar or grain; however this raw material base is not sufficient enough to support future large scale demand hence, bioethanol will most certainly have to be based on production from lignocellulosic material. There is copious amount of lignocellulosic biomass worldwide that can be exploited for fuel ethanol production. Significant advances have been made at bench scale towards the cellulosic ethanol. However there are still technical and economical hurdles which make bioethanol programme unsuccessful. One of the major challenges is to reduce the enzyme cost and to optimize and speed up enzyme engineering and fermentation technology. The government policies needed to overcome the perceived risk of first application, if we are to realize its social benefits soon. Cost below those for fossil sources are foreseeable, with advances in pretreatment, enzyme production and enzymatic hydrolysis-the steps that overcome the natural resistance of plants to biological breakdown-offering by far the greatest economic leverage. This review gives an overview on status of bioethanol production technologies and research efforts that have been tried to reduce cost, to improve enzymatic hydrolysis and fermentation yield and to bring lignocellulosic ethanol towards industrial production.**

Keywords: Bioethanol; lignocellulose; fermentation; pyrolysis; severity index; phenoxy radicals

I. INTRODUCTION

The consumption of energy has increased steadily over the last century with the increased world population. Fossil fuels are the major resource of energy where coal and oil supplies around 97% of the energy consumed for transportation, industry, heating purposes etc. The major fraction of oil i.e. about two third part goes to transportation. The intensive utilization of petroleum and oil has led to increase in the concentration of greenhouse gases (Carbon dioxide). This increased concern for security of oil supply and negative impact of fossil fuel on environment particularly global warming has put pressure on modern society to find renewable fuel alternative. One of the most promising renewable solutions is the use of solar energy in the form of biomass. The global potential of bioenergy is represented in energy crops and lignocellulosic residues. Conversion of these feed stocks into biofuel is an important choice for the production of alternative energy source and reduction of polluting gases. In addition the utilization of biofuels has important economic and social effects. Growing of energy crops to biofuel production would imply a boost to agriculture sector.

Ethanol is one of the most employed liquid biofuel. Ethanol is an oxygenated fuel that contains 35% oxygen which emits reduced particulate and NO_X after combustion and has clean burning characteristics. It has higher octane number, broader flammability limit, higher heat of vaporization which allows for a higher compression ratio, shorter burn time and increased efficiency of internal combustion engine. Being an excellent transportation fuel ethanol can be used as blend with gasoline, 10% and 85% blends are being used in US and Brazil. Related to MTBE, ethanol has great octane booster properties; it is not toxic and does not contaminate water resources. Many countries have implemented or are implementing programs for addition of ethanol to gasoline. Government of India through a notification dated Sept 2002 made 5% ethanol blending mandatory in petrol in 9 states and three union territories. In the next phase i.e. by 2015 the supply of ethanol blended petrol would be extended to whole country and efforts would be made to increase the blending percentage to 10 %. Fuel ethanol production has increased remarkably because many countries are looking for reducing oil imports, boosting rural economies and improving air quality. The world ethyl alcohol production has reached about 51,000 million liters, USA and Brazil being the top producers. Ethanol is mainly produced from sugar or starch; however this raw material base which also has to be used for animal feed and human need ,will not be sufficient to meet the increased demand for fuel ethanol . Consequently, future large scale use of ethanol will have to be based on production from lignocellulosic material. The main focus of this review is to highlight significant aspects of lignocellulosic ethanol and provide an overview of current status of bioethanol production technologies including pretreatment, use of lignolytic enzyme; solid state fermentation processing and finally looking at cost of enzyme and potential of modern approaches which could be employed to reduce cost.

II. LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass such as agriculture residue, wood and energy crop is an attractive material for bioethanol production, since it is the most abundant reproducible resource on the earth. They are such abundant and inexpensive resource that existing supplies could support the sustainable production of liquid transportation fuel and also the feedstock is not directly related to food production. There are about 73.9Tg of dry crop waste in the world that could potentially produce 49.1Gl per year of bioethanol, about 1.5pg per year of dry lignocellulosic biomass from these wasted crops are also available for conversion to ethanol. The potential bioethanol production could replace 32% of global gasoline. Asia is the largest producer of bioethanol from crop residue and wasted crops, and could produce up to 29.1Gl per year of bioethanol [1]. Rice straw, wheat straw, rice husk and corn stover are the most favorable bioethanol feed stocks. The massive utilization of fuel ethanol in the world requires that its production technology must be cost effective and environmentally sustainable. Over the past decade the total cost of ethanol has dropped from more than $1.0/l to $0.3 to 0.5/l, with a projected cost of less than $0.25/l in the near future. Efficient utilization of the hemicellulose component of lignocellulosic feed stock offers an opportunity to reduce the cost of producing fuel by 25%.

Thus Lignocellulosic material represents a promising option as a feedstock for ethanol production considering their great availability, high yield, low cost and their low environmental impacts, but the main limiting factor is the high degree of complexity inherent to the processing of feed stock. This is related to composition and nature of lignocellulosic biomass. The basic structure of lignocellulosic biomass consists of three basic polymers: cellulose, hemicellulose and lignin. Cellulose and hemicellulose are macromolecule from different sugar whereas lignin is an aromatic polymer synthesized from phenyl propanoid precursor. Lignin is linked to both hemicellulose and cellulose forming a physical seal around the latter two components and it act as barrier preventing the penetration of solution and enzymes. Lignin is most recalcitrant to degradation whereas cellulose is more resistant to hydrolysis than hemicellulose because of its highly ordered crystalline structure.

Composition and percentage of these polymers vary from one plant species to another. Moreover the composition within single plant varies with age, stage of growth and other conditions. The bioconversion of cellulose and hemicellulose to monomeric sugar is harder to accomplish than the conversion of starch, presently used for bioethanol production. One of the advantages of bioconversion with lignocellulosics 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 applications. Processing of lignocellulosics to bioethanol consists of four major unit operations: Pretreatment, hydrolysis, fermentation, and product separation.

III. PRETREATMENT

The first step in bioconversion of lignocellulosic biomass to ethanol is pretreatment. It is an important tool for practical cellulose conversion process. Lignocellulosic complex is made up of matrix of cellulose and lignin bounded by hemicelluloses chains. During the pretreatment this matrix should be broken down in order to reduce the crystalline degree of cellulose. Thus the main aim of pretreatment is the removal of lignin and hemicellulose, reduction of crystalline cellulose and increase in the porosity of material. Additionally the pretreatment should improve the formation of sugars and avoid the formation of inhibitors. The yield of cellulose hydrolysis is less than 20% of theoretical when pretreatment is not carried out whereas the yield after pretreatment often exceeds 90% of the theoretical. A successful pretreatment must thus (1) improve formation of sugar or ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrates; (3) avoid the formation of byproduct inhibitory to the subsequent hydrolysis; and (4) be cost effective [2]. Nowadays for the pretreatment of lignocellulosics several physical, physical chemical, chemical and biological processes have been proposed and developed.

A. Physical Method

Lignocellulosic biomass can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. This reduction facilitates the access of cellulases to the biomass surface increasing the conversion of cellulose. Still they are unattractive due to their high energy and capital costs. Pyrolysis has also been tested as a physical method for pretreatment of lignocellulosic biomass. Cellulose rapidly decomposes when it is treated at high temperature to produce gaseous products and residual char.

B. Physical-Chemical Method

Physical-chemical pretreatment methods are considerably more effective than physical alone. The steam explosion is the most commonly used method. During this process, the use of saturated steam at high pressure causes auto hydrolysis reactions in which part of the hemicellulose and lignin is converted into soluble olygomers. The factors affecting steam explosion pretreatment are residence time, temperature, chip size and moisture content. To consider the combined action of both temperature and time over the performance of steam explosion pretreatment, the so called severity index has been defined including a correction term for this process if carried out under acidic conditions. A two-step steam pretreatment of soft wood by dilute acid impregnation acetic acid is generated from hydrolysis of acetyl group associated with hemicellulose and it may further catalyse hydrolysis and glucose or xylose degradation.

Pretreatment by water and steam alone in steam explosion process relies on release of natural acids from hemicellulose to break down the hemicellulose followed by rapid pressure release to quench the reaction and disrupts the fibrous structure. Thus hemicellulose is thought to be hydrolyzed by the acetic acid and other acids released during steam explosion pretreatment.

Addition of acid catalyst such as H_2SO_4 or SO_2 is a prerequisite to reach high sugar yield. It increases the recovery of hemicellulose sugar and improves the enzymatic hydrolysis of solid fraction and it also yield inhibitory substances. The advantage of steam explosion is low energy requirement compared to mechanical comminution and involves no recycling or environmental cost. It is considered as most effective option for hardwood i.e. poplar, birch, maple and agriculture residue but it is less efficient for softwood i.e. pine, cedar.

C. Ammonia Fiber/ Freeze Explosion

Ammonia fiber/freeze explosion (AFEX) pre-treatment involves liquid ammonia and steam explosion. In this process ground, pre-wetted lignocellulosic material at a moisture content of 15-30% is placed in a pressure vessel with liquid ammonia (NH_3) at a loading of about 1-2 kg NH_3/kg dry biomass. It is effective for the treatment of corn stover but against aspen chips, which contain higher lignin content than sugar cane bagasse, the AFEX process is less effective [3]. This system does not directly liberate any sugars, but allows the polymers to be attacked enzymatically to reduce to sugar.

D. Acid Pretreatment

Among all the pre-treatment methods, dilute acid pre-treatment was one of the most studied and widely used. Acid pre-treatments normally aim for high yields of sugars from lignocellulosic biomass. There are many types of acid pre-treatments including use of sulfuric acid, hydrochloric acid, peracetic acid, nitric acid, or phosphoric acid. Acid pre-treatment can utilize either dilute or concentrated acids to improve cellulose hydrolysis.

Dilute acid pre-treatments at moderate temperatures using either sulfuric or phosphoric acid are reported for lignocellulosic biomass, including the hemicellulose fraction, followed by enzyme catalyzed hydrolysis of the cellulosic fraction to glucose. There are primarily two types of dilute acid pretreatment processes: low solids loading (5-10% [w/w]), high-temperature (T4433 K), continuous-flow processes and high solids loading (10-40% [w/w], lower temperature (To433 K), batch processes [3]. In general, higher pretreatment temperatures and shorter reactor residence times result in higher soluble xylose recovery yields and enzymatic cellulose digestibility. Higher-temperature dilute acid pre-treatment has been shown to increase cellulose digestibility of pretreated residues. Depending on the substrate and the conditions used 80 and 95% of the hemicellulosic sugars can be recovered by dilute acid pre-treatment from the lignocellulosic feedstock.

Concentrated acids have also been used for pretreatment. A fuel ethanol production from cane baggase involving the pretreatment with concentrated sulphuric acid has been patented. Although acid pretreatment is powerful agent for cellulose hydrolysis but they also presents potential problems such as production of acid waste stream that must be reused or neutralized, formation of compounds such as acetic acid and furfural in hydrolysate which are toxic to microorganism, and need for corrosion resistant equipment..

E. Alkaline Pretreatment

Alkaline solutions can be used to pretreat lignocellulosic material and the effectiveness of pretreatment is dependent upon the lignin content of material. The characteristic of alkaline pre-treatment is that it can remove the lignin without having big effects on other components. NaOH treatment causes lignocellulosic biomass to swell, leading to an increase in the internal surface area, a decrease in the degree of crystallinity, and disruption of the lignin structure [4]. After treatment with the acid, an alkaline for example lime or soda treatment is needed to stop the acid activity. Alkali pre-treatment reduces the lignin and hemicellulose content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignin carbohydrate. Dilute NaOH is usually used for alkali pre-treatment. Considering economic and environmental aspects, dilute NaOH treatment would be much more suitable than the concentrated NaOH pre-treatment.

The digestibility of NaOH treated hardwood increased from 14-55% with decrease of lignin content from 65% to 20%. However no effect was observed on softwood with lignin content greater than 26%. Dilute NaOH pretreatment was also effective for the hydrolysis of straw with relatively low lignin content of 10-18%. Combination of dilute NaOH treatment and other treatments seems more efficient. Pretreatment of corn stover using dilute NaOH (2%) combined with irradiation (500 kGy) caused the glucose yield to increase from just 20% for NaOH pre-treatment to 43% [4]. Lime (calcium hydroxide) has been used to pre-treat wheat straw (358K for 3 h), poplar wood (423K for 6 h with 14-atm oxygen), switch grass (373K for 2 h), and corn stover (373K for 13 h). The major effect is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides.

F. Biological Pretreatment

In biological pretreatment processes, microorganisms such as brown, white and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials [5]. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials [6]. Hatakka [7] studied the pretreatment of wheat straw by 19 whiterot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks.

The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation [8]. Both enzymes have been found in the extracellular filtrates of many white-rot fungi for the degradation of wood cell walls [9]. Lip is a glycoprotein with a heme group in active centre. Its molecular mass ranges from 38 to 43 kDa and its pH vary from 3.3 to 4.7. It is most effective peroxidases that can oxidize phenolic and non-phenolic compounds, amines and aromatic ethers with appropriate ionization potential. MnP are molecularly very similar to LiP and are also glycosylated protein. They generate phenoxy radicals which in turn undergo a variety of reaction resulting in depolymerization. In addition MnP oxidizes non phenolic lignin model compounds.

Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin [10]. The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low

IV.HYDROLYSIS

As the pre-treatment is finished, the cellulose is ready for hydrolysis, meaning the cleaving of a molecule by adding a water molecule:

$(C_6H_{10}O_5)n + nH_2O$ ………………… $nC_6H_{12}O_6$.

This reaction is catalyzed by dilute acid, concentrated acid or enzymes (cellulase) and the latter has many advantages as the very mild conditions give high yields and the maintenance costs are low compared to alkaline and acid hydrolysis due to no corrosion problems. A number of processes for hydrolyzing cellulose into glucose have been developed over the years. The vast majority of processing schemes utilizes either cellulolytic enzymes or sulfuric acid of varying concentrations.

During acid hydrolysis, xylose is degraded rapidly to furfural and other condensation by products. These degradation products are inhibitory to microorganism. The biggest advantage of dilute acid processes is their fast rate of reaction but biggest disadvantage is their low sugar yield.

Enzymatic Hydrolysis

Enzymatic hydrolysis is generally considered to consist of three steps:

- The adsorption of cellulase enzyme onto the surface of cellulose.
- The subsequent breakdown of cellulose to fermentable sugar through the synergetic action of the cellulase enzyme.
- Desorption of the cellulase enzyme from the lignocellulosic residue into the supernatant.

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific. The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild condition and does not have a corrosion problem [11]. Both bacteria and fungi can produce cellulases 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 cellulases [12]. Cellulomonas fimi and Thermomonospora fusca have been extensively studied for cellulose production. As the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi [11]. Fungi that have been reported to produce cellulases include Sclerotium rolfsii, P. chrysosporium and species of Trichoderma, Aspergillus, Schizophyllum and Penicillium [11, 13]. Of all these fungal genera, Trichoderma has been most extensively studied for cellulase production [13].

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (EG, endo-1,4-D-glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-b-D-glucan cellobiohydrolase, or EC 3.2.1.91.) which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) β-glucosidase (EC 3.2.1.21) which hydrolyzes cellobiose to produce glucose. During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol.

In addition to the three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylesterase, xylanase, b-xylosidase, galactomannanase and glucomannanase [11].

Cellulase activity decreases during the hydrolysis. The irreversible adsorption of cellulase on cellulose is partially responsible for this deactivation. Addition of surfactants during hydrolysis is capable of modifying the cellulose surface property and minimizing the irreversible binding of cellulase on cellulose. The rate of enzymatic hydrolysis was improved by 33% using Tween 80 as a surfactant in the hydrolysis of newspaper. Wu and Ju [14] tested Pluronic F68 and F88 (BASF) and Tween 20 and 80 for enhancing the enzymatic hydrolysis of pretreated newsprint.

V. FERMENTATION

The sugar syrup obtained after cellulosic hydrolysis is used for ethanol fermentation. The ability to ferment pentoses along with hexoses is not widespread among microorganisms, *S. cereviseae* is capable of converting only hexose sugars to ethanol. The most promising yeasts that have the ability to use both C5 and C6 sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus*. However, ethanol production from sugars derived from starch and sucrose has been commercially dominated by the yeast *S. cereviseae*. Thermotolerant yeast could be more suitable for ethanol production at industrial level. In high temperature process energy savings can be achieved through a reduction in cooling costs. Considering this approach, many researchers developed solid state fermentation system for ethanol production from sweet sorghum and potato employing a thermotolerant *S. cereviseae* strain (VS3). Research is now focusing on developing recombinant yeast, which can greatly improve the ethanol production yield by metabolizing all form of sugars, and reduce the cost of operation. In this contention the researchers have made efforts by following two approaches. The first approach has been to genetically modify the yeast and other natural ethanologens additional pentose metabolic pathways. The second approach is to improve ethanol yields by genetic engineering in microorganisms that have the ability to ferment both hexoses and pentoses.

VI. SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

Generally, enzymatic hydrolysis and fermentation steps are performed separately, and the approach is known as separate hydrolysis and fermentation (SHF). It enables enzymes to operate at higher temperature for increased performance and fermentation organisms to operate at moderate temperatures, optimizing the utilization of sugars. The disadvantage of this method is inhibition of cellulose enzyme by glucose released during hydrolysis which calls for lower solids loading and higher enzyme loading to achieve reasonable yields. Lower sugar yields result in lower ethanol concentrations and thus increase the cost of fermentation and ethanol recovery.

The most important process improvement made for the enzymatic hydrolysis of biomass is the introduction of simultaneous saccharification and fermentation (SSF), which has been improved to include the co-fermentation of multiple sugar substrates. This approach combined the cellulase enzymes and fermenting microbes in one vessel. This enabled a one-step process of sugar production and fermentation into ethanol. Simultaneous saccharification of both carbon polymer, cellulose to glucose; and hemicellulose to xylose and arabinose; and, fermentation will be carried out by recombinant yeast or the organism which has the ability to utilize both C5 and C6 sugars. Adaptation of yeast to the inhibitors present in the medium is an important factor for consideration in the design of SSF process. SSF combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermenter does not inhibit cellulase action as much as high concentration of glucose; so, SSF is good strategy for increasing the overall rate of cellulose to ethanol conversion. SSF gives higher ethanol yield while requiring lower amounts of enzyme because end-product inhibition from cellobiose and glucose formed during enzymatic hydrolysis is relieved by the yeast fermentation. However, it is not feasible for SSF to meet all the challenges at industrial level due to its low rate of cellulose hydrolysis and most microorganisms employed for ethanol fermentation cannot utilize all sugars derived after hydrolysis. To overcome this problem, the cellulolytic enzyme cocktail should be more stable in wide range of pH and temperature. Also the fermenting microorganisms (yeasts or bacteria) should be able to ferment a wide range of C5 and C6 sugars.

VII. DIRECT MICROBIAL CONVERSION (DMC)

DMC is a method of converting cellulosic biomass to ethanol in which the enzymes involved in both hydrolysis and fermentation are produced by a single microorganism. The potential advantage of DMC is that a dedicated process step for the production of cellulase enzyme is not necessary. Cellulase enzyme production (or procurement) contributes significantly to the cost involved in enzymatic hydrolysis process. However, DMC is not considered the leading process alternative. This is because there is no robust organism available that can produce cellulases or other cell wall degrading enzymes in conjunction with ethanol with a high yield. Distinguishing features of *F. oxysporum* for ethanol production in comparison to other organisms are identified. These include the advantage of in situ cellulase production and cellulose fermentation, pentose fermentation, and the tolerance of sugars and ethanol. The main disadvantage of *F. oxysporum* is its slow conversion rate of sugars to ethanol as compared to yeast.

VIII. COST REDUCTION APPROACHES

Enzyme cost is considered to be a major impediment in extensive commercialisation of enzymatic cellulose hydrolysis. Over the past decade the total cost of ethanol has dropped from more than $1.0 to $0.3-0.5, with a projected cost of less than $0.25 in the near future. Efficient utilization of hemicellulose component of lignocellulosic feed stock offers an opportunity to reduce the cost of producing fuel ethanol by 25%. Most of the research efforts have been focused on lowering the cost of enzymes. Other investigators have looked at various aspects including aspects of using cheaper substrates, immobilizing enzymes, recycling enzymes and improvements related to process design. The approaches which have been considered in achieving the low cost fall in three groups.

(1) Screening for organisms with novel enzymes;

(2) Strain improvement of existing industrial organisms and enzyme engineering;

(3) Production and operations related factors such as choice of substrate, culturing conditions, recycling of enzymes and redesigning of processes.

A. Screening for Organisms with Novel Enzymes

In terms of enzyme novelty from an applications perspective, interest is focused on not only finding enzymes which could break down lignocellulose much more rapidly but also enzymes which could withstand pH, temperature and inhibitory agents more resiliently depending on the intended application. Mutant strains of *T. reesei* have been selected that produce extra cellular cellulases up to 35 g/l.

B. Strain Improvement of Existing Industrial Organisms and Enzyme Engineering

Genetic techniques have been used to clone the cellulose coding sequence into bacteria, yeast, fungi, to create new cellulose production system with possible improvement of enzyme production and activity. Unique lignocellulolytic enzymes with multiple activities can be created by artificial gene-fusion. A bifunctional exoglucanase-endoglucanase and a chimeric xylanases-endoglucanase were successfully constructed. Using a combination of genetic and DNA technology approaches, a few strains with increased specific activities have been reported.

IX. CONCLUSION

Fuels derived from cellulosic biomass are essential in order to overcome our excessive dependence on petroleum for liquid fuels and also addresses the buildup of greenhouse gases that cause global climate change. Although bioethanol production has been greatly improved by new technologies but there are still challenges that need further investigation. These challenges include maintaining a stable performance of genetically engineered microorganism in commercial scale and developing more efficient pretreatment technologies for lignocellulosic biomass. For a flourishing bioethanol industry government support is critical in correcting tax anomalies, exemption from excise and sales tax, deregulation of feedstock and its pricing, encouraging pilot projects and R&D work on bioethanol. Advances in pretreatment by acid catalyzed hemicellulose hydrolysis or employing an integrated approach in the form of consolidated bioprocessing with application of novel cocktails of enzyme for cellulose breakdown that can ferment all possible sugar to ethanol at high productivity are the major key factors to make bioethanol programme successful at commercial scale.

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