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Production of Bioethanol From Lignocellulosic Feedstock, As Raw Material Through 2 Step Enzymatic Process: An Alternative Energy Fuel

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ABSTRACT:- Energy is the lifeline of global economy and the diminishing fossil fuel reserves and increased concerns over environmental pollution accelerated the need to look for renewable and environmentally sustainable energy sources The production of ethanol utilizing lignocellulosic biomass feed stocks, generally, consists of four major unit operations: Pretreatment, Depolymerization or saccharification of holocellulose fraction, Fermentation of mixed free sugars to produce ethanol, Separation and purification. The difference in process steps between starch and lignocellulosic feed stocks is that lignocellulosic biomass requires a more complicated hydrolysis stage. The reason for this is that cellulose in the wood contains carbohydrate polymers called cellulose. Cellulose is made up of long chains of glucose and a more complex set of enzymes are required to break the long chains.Lignocellulosic biomass is a complex mixture of holocellulose (cellulose and hemi cellulose) carbohydrate polymers, lignin, and a smaller amount of other compounds generally known as extractives. This review sheds light on some of the practical approaches that can be adopted to make the production of lignocellulosic bioethanol economically attractive. These include the use of cheaper substrates, cost-effective pre-treatment techniques, overproducing and recombinant strains for maximized ethanol tolerance and yields, improved recovery processes, efficient bioprocess integration, economic exploitation of side products, and energy and waste minimization. Bioethanol produced from renewable biomass such as sugar, starch, or lignocellulosic materials, is expected to be one of the dominating renewable biofuels in the transport sector within the coming twenty years. Although the priority in global future in the ethanol production is put on lignocellulosic processing, this is considered as one of the most promising second- generation biofuel technologies.

Keywords:- Bioethanol, Biomass, Lignocellulosic ethanol, Fuel properties; Feedstock; Production; Bioconversion; Fermentation; Hydrolysis

I. INTRODUCTION

Current world economy runs on fossil fuels. This is especially true for the transport sector that accounts for around 20% of the total world delivered energy consumption and is dependent on petroleum fuels for 98% of its energy requirement.(1) Rising demand for energy in emerging countries, dependence on oil from politically unstable regions and expected fossil fuel shortages have made energy security an increasingly critical issue. In addition, the projected impacts of climate change are forcing governments to limit greenhouse gas (GHG) emissions.(2) Biofuels are an attractive alternative to current petroleum-based fuels as they can be utilized as transportation fuels with little change to current technologies and have significant potential to reduction for the same. Liquid or gaseous (methane or hydrogen) biofuels are derived from organic materials such as starch, oilseeds and animal fats, or cellulose. The other types of biomass-derived fuels under development are green diesel, cellulosic ethanol, butanol, pyrolysis liquids, diesel from algae, hydrocarbons from biomass Decades of research have demonstrated that biomass requires extensive processing--hydrolysis of the raw material into fermentable sugars, and its subsequent biological conversion into a myriad of fuels and chemicals.[3] Alternative lignocellulosic feedstocks include agricultural residues such as corn stover, wheat and rice straw and forestry residue; industrial residue such as pulp and paper processing waste; and energy crops such as switchgrass. But, unlike starch, which contains homogenous and easily hydrolyzed polymers, lignocellulose plant matter contains cellulose (23-53%),hemicellulose (20-35%), polyphenolic lignin (10-25%) and other extractable components. Apart from reducing the dependence on imported fuels, is paper aims to generate several other benefits like employment generation for the rural poor, regeneration of wastelands, reduction of emissions resulting from energy use that can lead to positive economic and environmental change.[4]

II. FEED STOCKS FOR ETHANOL PRODUCTION

Biofuels originate from plant oils, sugar beets, cereals,organic waste and the processing of biomass. Biological feedstocks that contain appreciable amounts of sugar—or materials that can be converted into sugar, such as starch or cellulose—can be fermented to produce bioethanol to be used in gasoline engines [5]. Bioethanol feedstocks can be conveniently classified into three types: (i) sucrose-containing feedstocks (e.g. sugar beet, sweet sorghum and sugarcane), (ii) starchy materials (e.g. wheat, corn, and barley),and (iii) lignocellulosic biomass (e.g. wood, straw, and grasses) [6].

Different feedstocks for bioethanol production and their comparative production potential.(Table-I)

Bioethanol production	potential (l/ton)
Sugar cane	70
Sugar beet	110
Sweet potato	125
Potato	110
Cassava	180
Maize	360
Rice	430
Barley	250
Wheat	340
Sweet sorghum	60
Bagasse and other cellulose biomass	280

The various techniques utilized for the conversion of lignocellulosic feedstock to ethanol are biochemical and thermo chemical by nature. But firstly the pretreatment of lignocellulosic feed stocks viz lignin is very necessary Because the feedstock can represent 440% of all process costs, and economical biomass-to-bioethanol process critically depends on the rapid and efficient conversion of all of the sugars present in both its cellulose and hemi cellulose fractions.[7]

III. BIOCHEMICAL CONVERSION

In biochemical conversion the plant fibre is separated into its component parts; cellulose, hemicelluloses, and lignin hence the term lignocellulosic or cellulosic ethanol.[8] The cellulose is then further broken down to simple sugars that are fermented to produce ethanol. Typically the process is carried out in 4 stages

1. Physical or chemical pretreatment of the plant fibres to expose the cellulose and reduce its crystallinity,
2. Hydrolysis of the cellulose polymer, with enzymes or acids, to simple sugars (glucose)

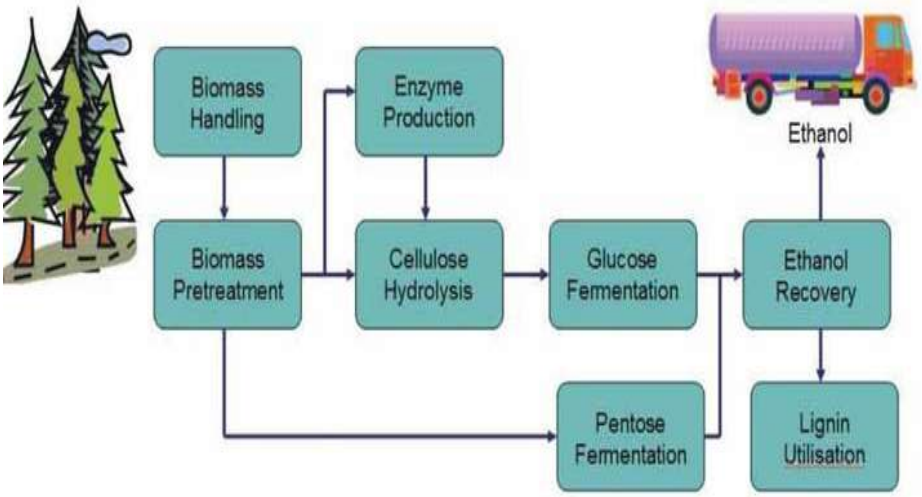


Fig-1 Biochemical Cellulose Ethanol Production

3. Microbial fermentation of these simple sugars to ethanol, and
4. Distillation and dehydration to produce 99.5% pure alcohol.([9])

Future process conversion efficiencies ;(Table-2)

Option	Fuel type	Process	Estimated efficiency improve-ment to 2020 (%)	2020 efficiency (GJ bioethanol/ GJ feedstock)
1	Bioethanol	Wood-acid hydrolysis	+5%	0.49
2	Bioethanol	Straw- acid hydrolysis	+5%	0.42
3	Bioethanol	Wheat	+10%	0.59
4	Bioethanol	Corn-wet milling	+20%	0.67
5	Bioethanol	Corn-dry milling	+20%	0.66
6	Bioethanol	Sugarcane	0	0.38
7	Bioethanol	Sugar beet	+5%	0.13

Table 2 summarises the data available for 2002 on process conversion efficiencies (product yields) for the developed biodiesel and bioethanol pathways [10]. Efficiencies are expressed in original units from the literature and as GJ biofuels per GJ feedstock. It shows projected improvements in process conversion efficiencies[11] for 2020 in comparison to 2002.

IV. THERMO CHEMICAL CONVERSION

Thermo chemical conversion transforms the lignocellulosic feedstock into carbon monoxide and hydrogen (syngas) by partial combustion. These gases can be converted to liquid transportation fuels or commodity chemicals by catalytic or biological pathways. The biological process converts carbon monoxide to ethanol using a non-yeast fermentation microorganism (eg. *Clostridium ljungdahlii*).[12] Alternatively, the syngas can be fed to a catalytic reactor where the carbon monoxide and water are combined via a metal-catalysed process to produce methanol, ethanol, other higher alcohols or liquid fuels (Fischer-Tropsch liquids).[13] Gasification is important because lignin, which constitutes about 25 – 30% of cellulosic biomass, is also converted to syngas and subsequently converted to fuel.[14]

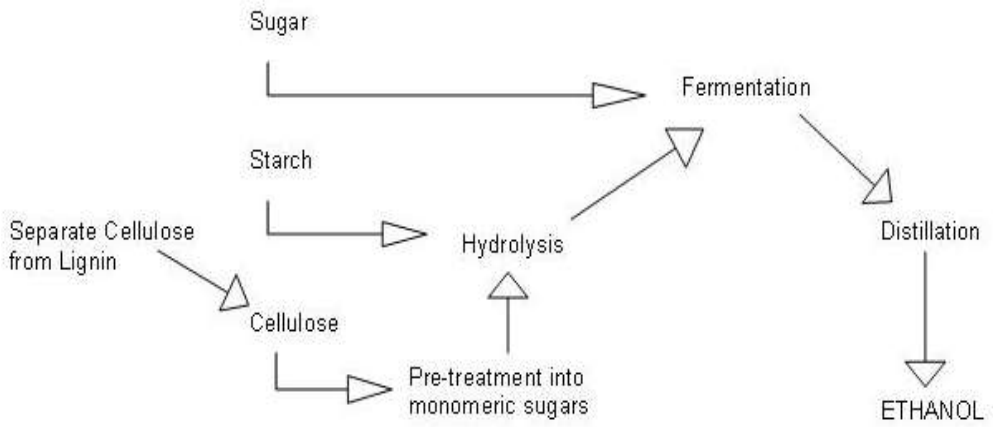


Fig-2 flow sheet of bioethanol production

Pre-treatment

The first step in bioconversion of lignocellosics to bioethanol is size reduction and pre-treatment. The goal of any pre-treatment 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 hemi cellulose [15] Pre-treatment is an important tool for practical cellulose conversion processes. Pre-treatment is required to alter the structure of cellulosic biomass to make more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars and to cellulose producing microorganisms .[16] Pre-treatment can be carried out in different ways such as mechanical pre-treatment, steam explosion, ammonia fiber explosion, supercritical CO2 treatment, alkali or acid pre-treatment. The ideal pretreatment liberates hemi

cellulose, exposes the cellulose and allows the lignin to be separated and must also minimize the formation of degradation products that can inhibit the subsequent hydrolysis and fermentation processes.[17]

Hydrolysis

After pretreatment there are two distinct processes to depolymerize holocellulose fraction into monomeric sugars namely: acid hydrolysis and enzymatic hydrolysis.[18] The basic hydrolysis reactions of 5 carbon (xylose and arabinose) and 6 carbon sugars (glucose, galactose, and mannose) are presented below.

$(C_5H_8O_4)n + n\ H_2O \rightarrow n\ C_5H_{10}O_5$ ----------(1)

$(C_6H_{10}O_5)n + n\ H_2O \rightarrow n\ C_6H_{12}O_6$ ----------(2)

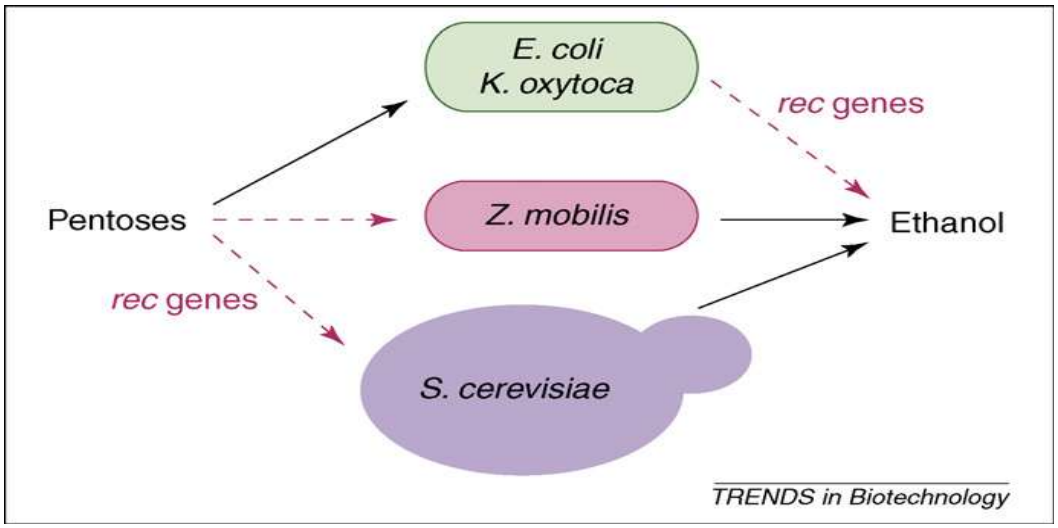
Acid Hydrolysis

The acid hydrolysis is mainly performed through dilute acid and concentrated acid hydrolysis. In this process if higher temperatures or longer residence times are applied, the monosaccharide formed from hemi cellulose will undergo degradation giving rise to fermentation impediments such as furan compounds, weak carboxylic acids, and phenolic compounds. 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 [19]: 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 monosaccharide 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 [20] of more resistant cellulose fraction into glucose. In **Concentrated Acid Hydrolysis** depolymerization of holocellulose followed by a dilution with water to dissolve the substrates into sugar constituents. This process enables complete and rapid conversion of cellulose to glucose and hemi cellulose to xylose with a little degradation The primary advantage of this process is its high sugar recovery efficiency; about 90% of both hemi cellulose and cellulose fraction gets depolymerize into their respective monomeric sugars.

Enzymatic Hydrolysis

Enzymatic hydrolysis of natural lignocellulosic materials is a very slow process because cellulose hydrolysis is hindered by structural parameters of the substrate, such as lignin and hemicellulose content, surface area, and cellulose crystallinity. During the enzymatic hydrolysis of cellulosic substrates, several factors restrict the sustained catalytic activity of the cellulase mixture. It has been suggested that these limitations are owing to both substrate- and enzyme-related factors [21]. It has been difficult to evaluate the reuse and/or recycle of cellulases, primarily because our current knowledge of the characteristics of cellulase adsorption onto lignocellulosic substrates is insufficient [22]. The enzymatic degradation of solid cellulose is a complicated process that takes place at a solid–liquid phase boundary, where the enzymes are the mobile components . When cellulase enzyme systems act in vitro on insoluble cellulosic substrates, three processes occur simultaneously [23]: (i) chemical and physical changes in the residual (not yet solubilized) solid-phase cellulose, (ii) primary hydrolysis, involving the release of soluble intermediates from the surface of reacting cellulose molecules, and (iii) secondary hydrolysis, involving hydrolysis of soluble intermediates to lower molecular weight intermediates, and ultimately to glucose. The rate of enzymatic hydrolysis of the cellulosic materials always decreases rather quickly. Generally, enzymatic cellulose degradation is characterized by a rapid initial phase followed by a slow secondary phase that may last until all substrate is consumed. Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. [24]

Acid-catalyzed pretreatment primarily solubilizes the hemicellulose fraction into the liquid phase. For softwood, the liquid mainly contains solubilized mannose in addition to small amounts of xylose, arabinose, galactose glucose.The solid phase comprises lignin and cellulose, the latter of which is subjected to enzymatic hydrolysis. The maximum cellulase activity of most fungal-derived cellulases and bglucosidases is observed at _50 8C and at a pH of 4.0–5.0; however, the optimal conditions vary with the hydrolysis time and are dependent on the source of the enzymes. Cellulases belong to two groups of enzymes known as endoglucanases (EG) and cellobiohydrolases (CBH), respectively. EG randomly attack the cellulose chain, creating free ends for CBH to cleave dimers of glucose (cellobiose) off. A third type of enzyme, b-glucosidase, which hydrolyzes cellobiose into two glucosemolecules, is also necessary: in the absence of b-glucosidase, end-product inhibition from cellobiose will occur. Furthermore, compounds generated during pretreatment might have an adverse effect on enzymatic hydrolysis. The enzymatic hydrolysis of spruce was greatly improvedwhen the liquid fraction fromthe pretreatment step was replaced with a buffer solution . This could not be entirely ascribed to the reduction in end-product inhibition, suggesting that inhibitory compounds had also been removed



V. FERMENTATION

Separate hydrolysis and fermentation (SHF)

In this system the substrate flow from the pretreatment and hemicellulose hydrolysis is subjected to enzyme hydrolysis by the cellulose enzyme complex. Then the flow enters the glucose fermentation reactor. The mixture is after that distilled to remove the ethanol. In a second reactor the xylose is fermented to ethanol, and the ethanol is again distilled. The cellulase production uses substrate from the hemicellulose hydrolysis.

Simultaneous saccharification and fermentation (SSF)

Current strategies to produce fuel ethanol from cellulose, referred to as "second-generation" biofuels, utilize

simultaneous saccharification and fermentation (SSF) or **simultaneous saccharification and co-fermentation** (SSCF) Both SSF and SSCF require extensive pretreatment of the cellulosic feedstock by steam-explosion and/or acid treatment, followed by addition of exogenously produced cocktails of cellulolytic enzymes to hydrolyse cellulose chains and release the glucose monomers required for fermentation.

Simultaneous saccharification and co-fermentation (SSCF)

This process represents hydrolysis of the cellulose and co-fermentation of pentose and hexose sugars by xylose- and glucose-fermenting microorganisms in one vessel. Cellulase is produced separately using a hemicellulose hydrolysate. The microorganisms are genetically engineered. Progress is rapid in the field of xylose fermentation, but few industrial yeast strains have yet the demonstrated capability of fermenting xylose in lignocellulosic hydrolyzates efficiently.[25]

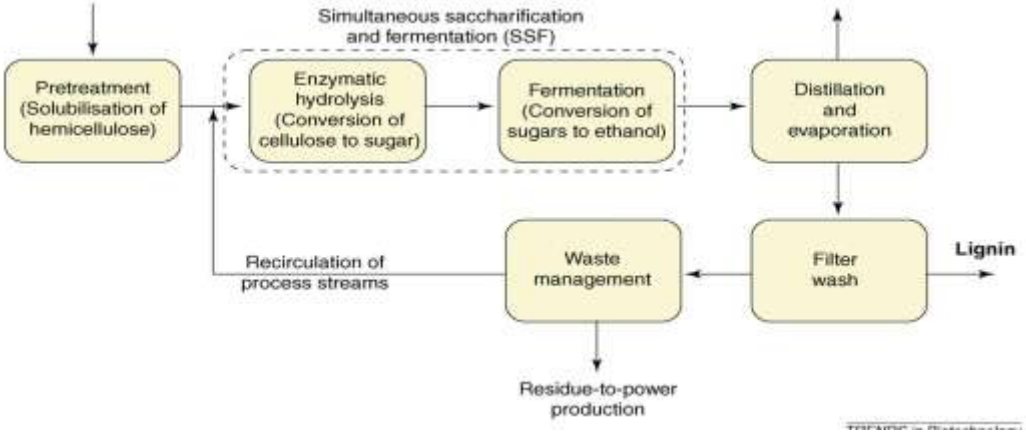
Simultaneous saccharification and fermentation

(SSF) is a process option for production of ethanol from lignocellulose and consolidates hydrolysis of cellulose with the direct fermentation of the produced glucose. Both hydrolysis of cellulose by the cellulase complex and fermentation of hexoses by the ethanologenic microorganism are coupled in one vessel . The pentoses are fermented before the hydrolysis of cellulose in a separate fermenter. Cellulase is produced in a separate fermentor using hemicellulose hydrolysate. The principal benefits of performing the enzymatic hydrolysis together with the fermentation, instead of in a separate step after the hydrolysis, are the reduced end-product inhibition of the enzymatic hydrolysis, and the reduced investment costs. SSF is today important in the dry-milling process in the corn-based ethanol industry in the US. The yield from the SSF are in the range of 80–85% on the basis of total carbohydrates. The simplest – and original – SSF is a batch process in which substrate, enzymes and yeast are all present in the reactor initially, and at the intended concentrations. Enzymatic hydrolysis has to be improved in order to reduce the cost of consumption of the enzymes . Research works will have to focus upon the enzyme specific activity, in order to achieve higher efficiencies. The SSF process improves the enzyme efficiency by reducing the feed-back inhibition from the hydrolysis products. Combining cellulose hydrolysis and glucose fermentation in one vessel could improve rates, yields, concentrations. The big benefit of SSF is that it reduces sugar inhibition to enzymes, realizing high-solids fermentation, improved cellulose conversion rates, increased ethanol concentration, low enzyme loadings. The screening of efficient fermentative microorganisms under high temperature conditions has to be further implemented because the optimal saccharification temperature is 45°C, and the optimal fermentation temperature is 30°C

VI. MICROORGANISMS

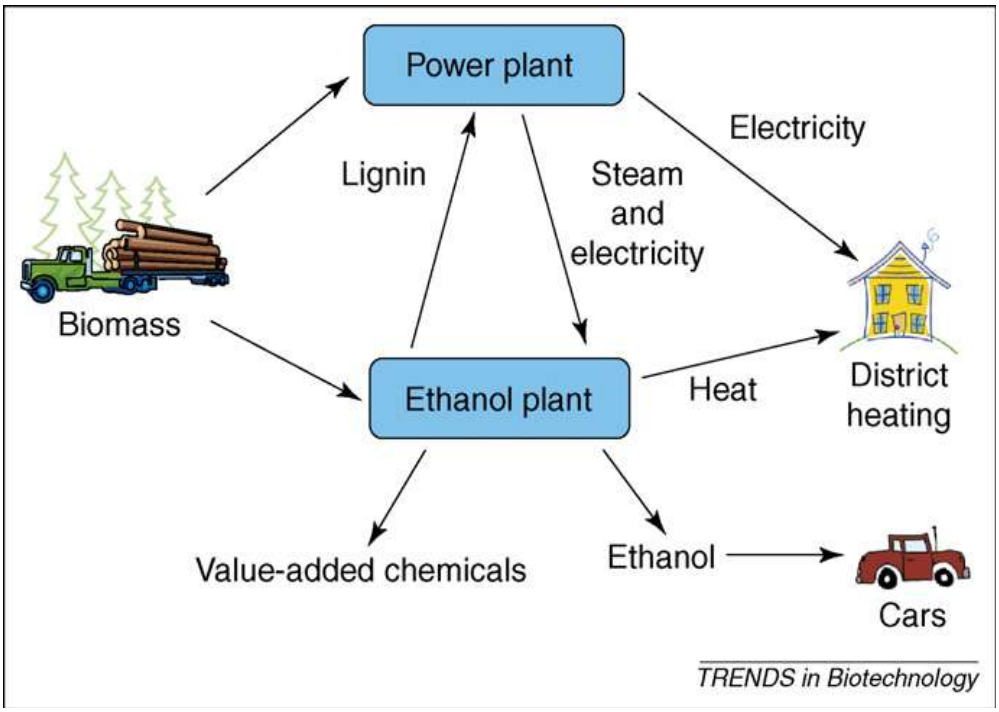
The industrial fermentation of lignocellulose hydrolysate to ethanol requires microorganisms, which have a broad substrate range, and which produce ethanol with high yield and productivity. Such microorganisms must also tolerate ethanol and inhibitors formed in the pretreatment process. Most research efforts have been devoted to the development of efficient xylose-fermenting microorganisms.

Two groups of microorganisms - enteric bacteria and some yeasts - are able to ferment pentoses, but with low ethanol yields. Furthermore, in the case of xylose fermenting yeasts (*Pachysolen tannophilus, Candida shehatae*, and *Pichia stipitis*), large-scale utilization is



Process diagram for the conversion of cellulose/hemicelluloses to ethanol

Hampered by their sensitivity to high concentrations of ethanol (≥40 g/l), the requirement for carefully monitored microaerophilic conditions, high sensitivity to inhibitors, and the inability to ferment xylose at low pH. Lignocellulosic raw materials, in particular hardwood andagricultural raw materials, can contain 5–20% (or more) of the pentose sugars xylose and arabinose, which are not fermented to ethanol by the most commonly used industrial fermentation microorganism, the yeast Saccharomyces cerevisiae. Xylose is by far the most abundant pentose sugar, whereas arabinose can constitute as much as 14–15% in corncob hulls and wheat bran, respectively. Consequently, most research efforts have been devoted to the development of efficient xylose-fermenting microorganisms. Xylose-fermenting microorganisms are found among bacteria, yeast and filamentous fungi Anaerobic bacteria ferment pentoses, but are inhibited already at low sugar and ethanol concentrations. In addition, the ethanolic fermentation occurs with considerable by-product formation, which reduces the ethanol yield. Natural xylose-fermenting yeast, notably Pichia stipitis CBS 6054, ferment xylose to ethanol with reasonable yield and productivity; however, these yeast strains are inhibited by compounds generated during pretreatment and hydrolysis of the lignocellulose material . Filamentous fungi tolerate inhibitors but are too slow for a competitive industrial process. Therefore, efforts have predominantly been made to obtain recombinant strains of bacteria and yeast able to meet the requirements of industrial lignocellulose fermentation Pentose-fermenting Escherichia coli and Klebsiella oxytoca have been generated by introducing ethanologenic genes from Zymomonas mobilis . At the same time, the first xylose-fermenting S. cerevisiae strain was generated through the introduction of genes for xylose metabolizing enzymes from P. stipitis . Later xylose-fermenting strains of S. cerevisiae were constructed by introducing the genes encoding xylose isomerase from the bacterium Thermus thermophilus and the anaerobic fungus Piromyces sp. respectively. For xyloseusing S. cerevisiae, high ethanol yields from xylose also require metabolic engineering strategies to enhance the xylose flux. . Z. mobilis also efficiently produces ethanol from the hexose sugars glucose and fructose but not from pentose sugars, although a xylose fermenting Z. mobilis was generated by introducing a xylose-metabolizing pathway from E. coli . More recently, the obligatory anaerobic bacterium Thermoanaerobacterium saccharolyticum has been genetically engineered for improved ethanolic fermentation (Joe Shaw et al., oral presentation, Nashville, 2006). E. coli and K. oxytoca naturally metabolize arabinose, such that the ethanologenic strains ferment all lignocellulose- derived sugars ; furthermore, xylose- and arabinose fermenting strains of Z. mobilis have been constructed . Because yeast only ferment arabinose to ethanol in rich media, S. cerevisiae has been engineered for arabinose use by introducing both bacterial and fungal genes encoding arabinose-metabolizing enzymes, where the fungal approach did not result in appreciable arabinose fermentation. The functional arabinose-metabolizing pathway has recently been integrated into the diploid xylose-fermenting S. cerevisiae strain TMB 3400, and co-usage of xylose and arabinose has been demonstrated.



Biorefinery – integration of a combined heat and power plant with an ethanol production plant.

VII. CONCLUSION

More recently, the SSF technology has proved advantageous for the simultaneous fermentation of hexose and pentose sugars (so called 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. Further process integration can be achieved by performing both 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 further research

Production of bioethanol from biomass is one way to reduce both the consumption of crude oil and environmental pollution. Large amounts of CO_2 are released during corn bioethanol production contributing to the global warming problem. Using bioethanol-blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Bioethanol is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NOx emissions from combustion. Ethanol has a higher octane number (108), broader flammability limits, higher flame speeds and higher heats of vaporization. These properties allow for a higher compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in an ICE. Bioethanol is blended with gasoline to form an E10 blend (10% bioethanol and 90% gasoline), [30] but it can be used in higher concentrations such as E85 or E95. There are several options for a lignocelluloses-to-ethanol process but, regardless of which is chosen, the following features must be assessed in comparison with established sugar- or starch-based ethanol production.

(i) Efficient de-polymerization of cellulose and hemicellulose to soluble sugars.
(ii) Efficient fermentation of a mixed-sugar hydrolysate containing six-carbon (hexoses) and five-carbon (pentoses) sugars as well as fermentation inhibitory compounds.
(iii) Advanced process integration to minimize process energy demand.
(iv) Cost-efficient use of lignin.

The possibility of obtaining a renewable, available, safe and effective source of energy is one of the challenges that humanity should face. The biofuels, particularly the bioethanol, are an environmentally clean source of energy. An important part of the research trends on fuel ethanol production is oriented to the reduction of feedstock costs, especially through the utilization of less expensive lignocellulosic biomass. In general, most of the research efforts are oriented to the conversion of lignocellulosic into fermentable sugars and useful intermediates (due to the recalcitrance or resistance of the biomass to be converted). The key factor for enhancing the competitiveness of biomass-to-ethanol process is the increase in the specific activity of cellulases

and the decrease in their production costs. The potential for bioethanol to create jobs is immense in farming, biorefineries, the chemical industry, the fuel supply sector as well as fuel-flexible vehicle engineering. The economic climate is ripe for investing in bioethanol production in Europe mostly for fuel ethanol but also for the chemical use and stationary power generation. The bioethanol by-products provide a useful side revenue through feed stocks for animal feed, power generation and as a feedstock for 2nd generation bioethanol.

The importance of continuing cellulosic biomass process development investment cannot be understated. Although significant progress has been made, commercialization of lignocellulosic conversion to ethanol has been difficult, not only due to the heterogeneous nature of biomass itself, but also due to multiple treatments required for effective processing. It is quite apparent that development of advanced enzyme technologies is critical, as today's commercial cellulases are inadequate for cost-effective biomass processing. Successes from pilot projects have clearly demonstrated that understanding interaction between cellulase action and pretreatment can facilitate and accelerate progress in this area. Such integrated approaches are enabling superior multi-component cellulase systems to be developed. Further improvements can be achieved by integrating these processes with fermentation.

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