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Standard Review

Economics and environmental impact of bioethanol production technologies: an appraisal

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Contemporary industrial developments and rapid pace of urbanization have called for an environmentally sustainable energy sources. Ethanol made from biomass provides unique environmental, economic strategic benefits and can be considered as a safe and cleanest liquid fuel alternative to fossil fuels. There is a copious amount of lignocellulosic biomass worldwide that can be exploited for fuel ethanol production. Significant advances have been made at bench scale towards the fuel ethanol generation from lignocellulosics. However there are still technical and economical hurdles, which make the bioethanol program unsuccessful at commercial scale. This review provides a broad overview on current status of bioethanol production technologies in terms of their economic and environmental viability. These technologies include pretreatment of biomass, the use of cellulolytic enzymes for depolymerisation of carbohydrate polymers into fermentable constituents and the use of robust fermentative microorganisms for ethanol production. Among all the available technologies, dilute acid hydrolysis followed by enzymatic hydrolysis by less expensive and more efficient cellulases has been found more promising towards the potential economics and environmental impact.

Key words: Bioethanol, lignocellulosic feedstock, ethanol fermentation, economics, environmental impact.

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INTRODUCTION

In 1925, Henry Ford had quoted ethyl alcohol, ethanol, as "the fuel of the future." He furthermore stated, "The fuel of the future is going to come from apples, weeds, sawdust – almost anything. There is fuel in every bit of vegetable matter that can be fermented." Today Henry Ford's futuristic vision significance can be easily understood.

In the current time, the importance of alternative energy source has become even more necessary not only due to the continuous depletion of limited fossil fuel stock but also for the safe and better environment, with an inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy (Wyman, 1999; Lynd, 2004; Herrera, 2004; Herrera, 2006; Lin and Tanaka, 2006, Schubert, 2006; Chandel et al., 2006a; Vertes et al., 2006, Dien et al., 2006). Keeping in view all the above said advantages, biomass based fuel development technologies should rapidly gain momentum and the barriers imposed earlier should be removed for successfully attempting the production of bioethanol at the commercial level.

It is welcome to understand that the use of bioethanol as a source of energy would be more than just complementting for solar, wind and other intermittent renewable energy sources in the long run (Lin and Tanaka, 2006). During the last two decades, advances in technology for ethanol production from biomass have been developed to the point that large-scale production will be a reality in next few years (Yu and Zhang, 2004; Moiser et al., 2006). Ethanol production from biomass can be summarized briefly into following steps: depolymerization of holocellulose polymer into monomeric fermentable substrate, fermentation of depolymerized substrates, and the distillation of the fermentation broth to obtain dehydrated ethanol.

The ethanol yields and processes economics along with the technical maturity and environmental benefits of using ethanol blend fuel are the key parameters that determine the feasibility of bioethanol production (Nguyen and Saddler, 1991).

The burning fossil fuel at the current rate is likely to create an environmental crisis globally. Use of fossil fuel generates carbon dioxide, methane and a significant quantity of nitrous oxide. Most of these harmful gases are formed due to incomplete combustion of fossil fuel; since ethanol contains 35% oxygen that may result in a more complete combustion of fuel and thus reduces tailpipe emissions.

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Moreover, biomass energy can play an important role in reducing green house gas emissions. Ethanol production process only uses energy from renewable energy sources. Hence no net carbon dioxide is added to the atmosphere, making ethanol an environmentally bene-ficial energy source (Bull et al., 1992; Kheshgi et al., 2000). Furthermore, fuel ethanol from lignocelluloses may also open new employment opportunities in rural areas, and thus make a positive socio-economic impact (Wyman, 2003; Bevan and Franssen, 2006). Developing ethanol as fuel, beyond its current role as fuel oxygenates will require developing lignocellulosic biomass as a feedstock because of its abundantly available and low cost.

The world ethanol production in 2004 was estimated to be 40 giga litres (GL) (Berg, 2004; Kim and Dale, 2004). Brazil and the US are the world leaders, which together accounted for about 60% of the world ethanol production exploiting sugarcane and corn respectively. In India, lignocellulosic biomass (crop residues, forestry and fruit and vegetable waste and weeds) is available in plenty. Renewable fuels particularly ethanol should get more and more attention all over the world.

The important issue that we wish to address affirmatively here is that the bioethanol production, without doubt, needs an economical approach to address the global fuel needs. Research efforts are needed to design and improve the process, which would produce sustainable and economically feasible transportation fuel. Improvement in process economics using new designed cellulases enzyme cocktail are important factors in establishing a cost effective technology, besides the low cost of feedstock (Mojovic et al., 2006; Gray et al., 2006).

For the long haul, it is very important to understand bioethanol production technologies in terms of their economic viability, environmental feasibility and empowering employment opportunities before implementing a fuel ethanol policy.

The choice of the best technology for lignocellulose to bioethanol conversion should be decided on the basis of overall economics (lowest cost), environmental (lowest pollutants) and energy (higher efficiencies)that is, comprehensive process development and optimization are still required to make the process economically viable.

In reality, environmental considerations, energy and tax policies will determine the extent of fuel ethanol utilization in the future (Keim and Venkatasubramanian, 1989) and therefore the role of one and all is very crucial to identify the gravity of the situation associated with bioethanol production and use of it as an alternative fuel.

The focus of this review is on the current status of available ethanol production technologies in terms of their

practical cost economics and the desirable environmental impact they have for a whole generation of commuters across the globe.

Lignocellulosic biomass

Composition

Basically, the lignocellulosic biomass comprises of cellulose, hemicellulose and lignin (Hayn et al., 1993). Cellulose is a linear, crystalline homopolymer with a repeating unit of glucose strung together beta-glucosidic linkages. The structure is rigid and harsh treatment is required to break it down (Gray et al., 2006). Hemi-cellulose consists of short, linear and highly branched chains of sugars. In contrast to cellulose, which is a polymer of only glucose, a hemicellulose is a hetero-polymer of D-xylose, D-glucose, D-galactose, D-man-nose and L-arabinose (Saha et al., 2003). The composi-tion of holocellulose (cellulose + hemicellulose) varies with the origin of the lignocellulosic material. Table 1 shows the composition of the selected crop residues, woody materials, vegetable and fruit waste and municipal solid waste and their simulated ethanol production.

Ethanol production has been taken into estimation depending upon the ratio of hexosans (glucan, galactan and mannan) and pentosans (xylan, arabinan) in each bio-mass source. The ethanol production from each bio-mass sources was calculated / ascertained from US Department of Energy website, which provide “Theoretical ethanol yield calculator” at http://www.eere.ene-rgy.gov/biomass/ethanol_yield_calculator.html.

Ethanol production technologies

Bioconversion of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation and product separation/ distillation.

Pretreatment

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields (Sun and Cheng, 2002; Moiser et al., 2005). Pretreatment affects the structure of biomass by solubilizing hemicellulose, reducing crystallinity and increase the available surface area and pore volume of the substrate. Pretreatment has been considered as one of the most expensive processing step in biomass to fermentable sugar conversion with cost as high as 30 cents/gallon ethanol produced (Moiser et al., 2005). To asses the cost and performance of pretreatment methods, technoeconomic analysis have been made recently (Eggerman and Elander, 2005). There is huge scope in lowering the cost of pretreatment process through extensive R&D approaches. Pretreatment of cellulosic biomass in cost effective manner is a major challenge of cellulose to ethanol technology research and development.

Native lignocellulosic biomass is extremely recalcitrant to enzymatic digestion. Therefore, a number of thermochemical pretreatment methods have been developed to improve digestibility (Wyman et al., 2005). Recent studies have clearly proved that there is a direct correlation between the removal of lignin and hemi-cellulose on cellulose digestibility (Kim and Holtzapple, 2006). Thermochemical processing options appear more promising than biological options for the conversion of lignin fraction of cellulosic biomass, which can have a detrimental effect on enzyme hydrolysis. It can also serve as a source of process energy and potential co-products that have important benefits in a life cycle context (Sheehan et al., 2003). Pretreatment can be carried out in different ways such as mechanical combination (Cadoche and Lopez, 1989), steam explosion (Gregg and Saddler, 1996), ammonia fiber explosion (Kim et al., 2003), acid or alkaline pretreatment (Damaso et al., 2004; Kuhad et al., 1997) and biological treatment (Keller, et al., 2003).

Hydrolysis

After pretreatment there are two types of processes to hydrolyze the feed stocks into monomeric sugar constituents required for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic. To improve the enzymatic hydrolytic efficiency, the lignin-hemicellulose net work has to be loosened for the better amenability of cellulases to residual carbohydrate fraction for sugar recovery. Dilute acid treatment is employed for the degradation of hemicellulose leaving lignin and cellulose network in the substrate. Other treatments are alkaline hydrolysis or microbial pretreatment with white-rot fungi (*Phaenerochate chrysosporium*, *Cyathus stercoreus*, *Cythus bulleri* and *Pycnoporous cinnabarinus* etc.) preferably act upon lignin leaving cellulose and hemicellulose network in the residual portion. However during both treatment processes, a considerable amount of carbohydrates are also degraded, hence the carbohydrate recovery is not satisfactory for ethanol production.

Acid hydrolysis

There are two types of acid hydrolysis process commonly used - dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or minutes. The concentrated acid process uses relatively mild temperatures, but at high concentration of sulfuric acid and a minimum pressure involved, which only creates by pumping the materials from vessel to vessel. Reaction times are typically much longer than for dilute acid

Table 1. Chemical composition of raw materials and simulated ethanol production

Raw material	Cellulose/ Hexosans (H)	Hemicellulose / Pentosans (P)	Lignin	Ethanol yield /kg dry mass	Reference
Sugarcane baggase	33 (H)	30 (P)	29	0.279	Kuhad and Singh, 1993
Wheat straw	30 (H)	24 (P)	18	0.239	Kuhad and Singh, 1993
Sorghum straw	33 (H)	18 (P)	15	0.240	Kuhad and Singh, 1993
Rice straw	32 (H)	24 (P)	13	0.248	Kuhad and Singh, 1993
Oat straw	41 (H)	16 (P)	11	0.252	Kuhad and Singh, 1993
Corn cob	42 (H)	39 (P)	14	0.358	Kuhad and Singh, 1993
Corn stalks	35 (H)	15 (P)	19	0.221	Kuhad and Singh, 1993
Barley straw	40 (H)	20 (P)	15	0.265	Kuhad and Singh, 1993
Ground nut shell	38 (H)	36 (P)	16	0.327	Kuhad and Singh, 1993
Alfalfa stalks	48.5	6.5	16.6	0.209	Shleser, 1994
Rice hulls	36 (H)	15 (P)	19	0.265	Kuhad and Singh, 1993
Eucalyptus grandis	38	13	37	0.225	Shleser, 1994
Eucalyptus saligna	45	12.0	25.0	0.252	Shleser, 1994
Pine	44.0	26.0	29.0	0.310	Olsson and Hagerdal, 1996
Poplar	47.6	27.4	19.2	0.332	Olsson and Hagerdal, 1996
Saw dust	55.0	14.0	21.0	0.305	Olsson and Hagerdal, 1996
Willow	37.0	23.0	21.0	0.265	Olsson and Hagerdal, 1996
Aspen	51	29.0	16.0	0.354	Olsson and Hagerdal, 1996
Spruce	43.0	26.0	29.0		Olsson and Hagerdal, 1996
Birch	40.0	23.0	21.0	0.305	Olsson and Hagerdal, 1996
Lantana camara	42.50	22.70	22.88	0.288	Chandel (Unpublished work)
Prosopis juliflora	45.5	20.38	24.65	0.291	Chandel (Unpublished work)
Saccharum spontaneum	45.10	22.70	24.56	0.300	Gupta, 2006
Eicchornia crassipis	18.2	48.7	3.50	0.296	Nigam, 2002
Paja brava	32.2	28.1	24.0	0.267	Sanchez et al., 2004
News Paper	61	16	21	0.341	Olsson and Hagerdal, 1996
Processed Paper	47	25	12	0.318	Olsson and Hagerdal, 1996
Paper-based municipal solid waste	43	13	6	0.248	Olsson and Hagerdal, 1996

process

Dilute acid hydrolysis

In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulosic fraction. Dilute sulfuric acid is mixed with biomass to hydrolyse hemicellulose to xylose and other sugars. Dilute acid is interacted with the biomass and the slurry is held at temperature ranging from120 - 220℃ for a short period of time. Thus hemicellulosic fraction of plant cell wall is depolymerised and will lead to the enhancement of cellulose digestibility in the residual solids (Nigam, 2002; Sun and Cheng, 2002; Dien et al., 2006; Saha et al., 2005). Dilute acid hydrolysis has some limitations. If higher temperatures (or longer residence time) are applied, the hemicelluosic derived monosaccharides will degrade and give rise to fermentation inhibitors like furan compounds, weak carboxylic acids and phenolic compounds (Olsson and Hahn- Hagerdal, 1996; Klinke et al., 2004; Larsson et al., 1999). These fermentation inhibitors are known to affect the ethanol production performance of fermenting microorganisms (Chandel et al., 2006b). In order to remove the inhibitors and increase the hydrolysate fermentability, several chemicals and biological methods have been used. These methods include overliming (Martinez et al., 2000), charcoal adsorption (Chandel et al., 2006b), ion exchange (Nilvebrant, 2001), detoxification with laccase (Martin et al., 2002; Chandel et al., 2006b), and biological detoxification (Lopez et al., 2004). The detoxification of acid hydrolysates has been shown to improve their fermentability; however, the cost is often higher than the benefits achieved (Palmqvist and Hahn- Hagerdal, 2000; von Sivers and Zacchi, 1996). Dilute acid hydrolysis is carried out in two stages- First-stage and two-stage.

First-stage dilute acid hydrolysis

The lignocellulosic material is first contacted with dilute

sulfuric acid (0.75%) and heated to approximately 50°C followed by transferring to the first stage acid impregnator where the temperature is raised to 190°C. Approximately, 80% of the hemicellulose and 29% of cellulose are hydrolyzed in the first reactor. The hydrolysate is further incubated at a lower temperature for a residence time of 2 h to hydrolyse most of the oligosaccharides into monosaccharides followed by the separation of solid and liquid fractions. The solid material again washed with plentiful of water to maximize sugar recovery. The separated solid material is sent to second stage acid hydrolysis reactor (Figure 1).

Two-stage dilute acid hydrolysis

In two-stage dilute acid hydrolysis process, first, biomass is treated with dilute acid at relatively mild conditions during which the hemicelluose fraction is hydrolyzed and the second stage is normally carried out at higher temperature for depolymerisation of cellulose into glucose. The liquid phase, containing the monomeric sugars is removed between the treatments, thereby avoiding degradation of monosaccharides formed (Figure 1). It is very important to avoid monosaccharide degradation products for improving the ethanol yield. Sanchez et al. (2004) carried out the two-stage dilute acid hydrolysis using Bolivian straw material, *Paja brava*. In first stage, *P. brava* material was pretreated with steam followed by dilute sulfuric acid (0.5 or 1.0% by wt) hydrolysis at temperatures between 170 and 230°C for a residence time between 3 and 10 min. The highest yield of hemicellulose derived sugars were found at a temperature of 190°C, and a reaction time of 5 – 10 min, whereas in second stage hydrolysis considerably higher temperature (230 °C) was found for hydrolysis of remaining fraction of cellulose.

Concentrated acid hydrolysis

This method uses concentrated sulfuric acid followed by a dilution with water to dissolve and hydrolyse the substrate into sugar constituents. This process provides complete and rapid conversion of celluose to glucose and hemicellulose to xylose with a little degradation. The concentrated acid process uses 70% sulfuric acid at 40 - 50°C for 2 to 4 h in a reactor. The low temperatures and pressure will lead to minimize the sugar degradation. The hydrolyzed material is then washed to recover the sugars.

In the next step, the cellulosic fraction has to be deploymerized. The solid residue from first stage is de-watered and soaked in 30 - 40% sulfuric acid for 50 min. at 100°C for further cellulose hydrolysis. The resulting slurry mixture is pressed to obtain second acid–sugar stream (approximately 18% sugar and 30% acid). Both the sugar steams from two hydrolysis steps are combined and may be used for subsequent ethanol production. Iranmahboob et al. (2002) performed the concentrated acid hydrolysis of mixed wood chips and found that maximum sugar recovery (78 - 82% of theoretical yields) was achieved at sulfuric acid concentration (26%) for 2 h of residence time.

The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency, about 90% of both hemicellulose and cellulose fraction gets depolymerized into their monomeric fractions. The acid and sugar syrup are separated via ion exchange and then acid is reconcentrated through multiple effect evaporators. The remaining lignin rich solids are collected and optionally palletized for fuel generation (Figure 2).

Enzymatic hydrolysis

The acid, alkaline or fungal pretreated lignocellulosics can be saccharified enzymatically to get fermentable sugars (Ghose and Bisaria, 1979; Kuhad et al., 1997; Itoh et al., 2003; Tucker et al., 2003). Bacteria and fungi are the good sources of cellulases, hemicellulases that could be used for the hydrolysis of pretreated lignocellulosics. The enzymatic cocktails are usually mixtures of several hydrolytic enzymes comprising of cellulases, xylanases, hemicellulases and mannanases. In the last decade, new cellulases and hemicellulases from bacterial and fungal sources have continued been isolated and regular efforts have been made for the improved production of enzymetic titers (Aro et al., 2005; Foreman et al., 2003). However, the cellulases were produced at a concentration too low to be useful. There is a group of microorganisms (*Clostridium*, *Cellulomonas, Tricho-derma*, *Penicillium*, *Neurospora*, *Fusarium*, *Aspergillus* etc.) showing a high cellulolytic and hemicellulolytic activity, which are also highly capable of fermenting monosaccharides. Genetic engineering is used to produce super strains, which are capable of hydrolysing cellulose and xylan along with fermentation of glucose and xylose to ethanol (Aristidou and Penttila, 2000; Lin and Tanaka, 2006). The utilization of cellulose by microorganisms involves a substantial set of fundamental phenomena beyond those associated with enzymatic hydrolysis of cellulose (Lynd et al., 2002).

Separate hydrolysis and fermentation (SHF)

Enzymatic hydrolysis performed separately from fermentation step is known as separate hydrolysis and fermentation (SHF) (Sreenath et al., 2001; Wingren et al., 2003). The separation of hydrolysis and fermentation offers various processing advantage and opportunities. It enables enzymes to operate at higher temperature for increased performance and fermentation organisms to operate at moderate temperatures, optimizing the utilization of sugars (Figure 3).

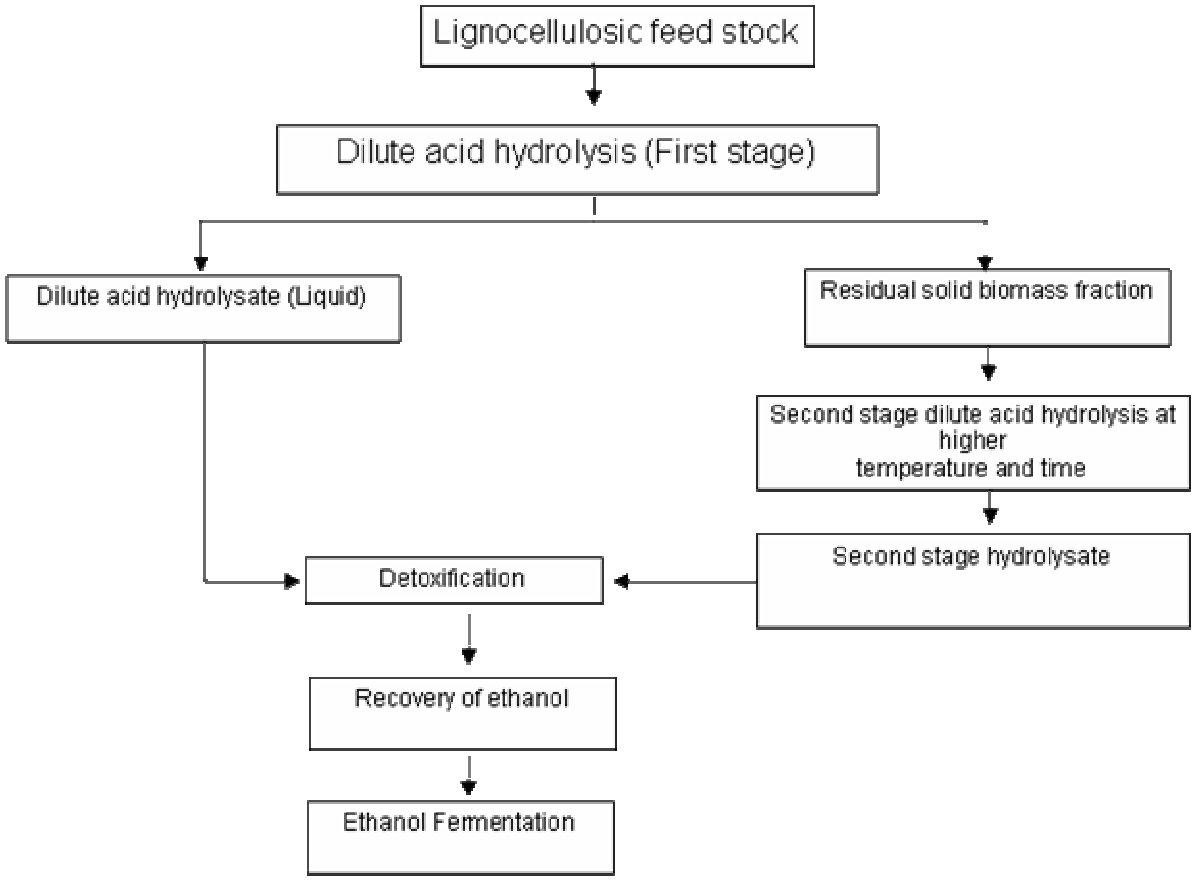

Figure 1. Dilute acid hydrolysis (First-stage and Two-stages) and separate fermentation of pentose and hexose sugars.

Simultaneous saccharification and fermentation (SSF)

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 (Figure 4) (Sreenath et al., 2001; Wingren et al., 2003). 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 C_5 and C_6 sugars. According to Alkasrawi et al. (2006) the mode of preparation of yeast must be carefully considered in SSF designing. A more robust strain will give substantial process advantages in terms of higher solid loading and possibility to recirculate the process stream, which results in increased energy demand and reduced fresh water utilization demand in process. Adaptation of yeast to the inhibitors present in the medium is an important factor for consideration in the design of SSF process. More recently, Kroumov et al. (2006) demonstrated an unstructured model of SSF of starch to ethanol by genetically modified strain *Saccharomyces cereviseae* YPB-G, using two hierarchic levels of concept. In first concept, a mechanism of enzymatic hydrolysis of starch to glucose by combined action of two enzymes (alpha-amylase and glucoamylase) secreted by recombinant yeast and the second concept was the enzymatic degradation of starch to glucose and simultaneous utilization of glucose to ethanol by microorganisms. 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 (Lin and Tanaka, 2006). 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 (Banat et al., 1998). However, it is not feasible for SSF to

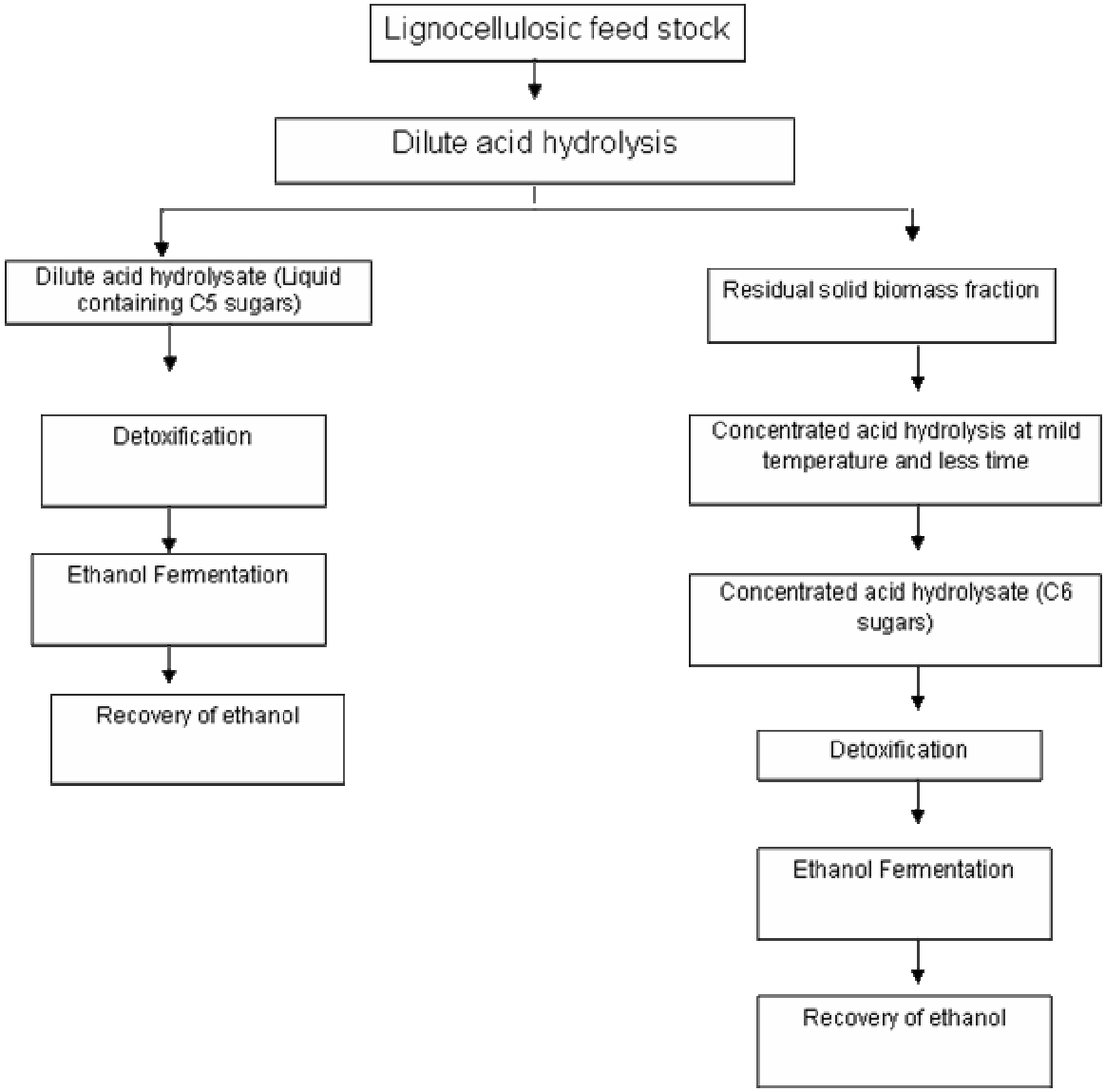

Figure 2. Concentrated acid hydrolysis and separate pentose and hexose sugars fermentation.

meet all the challenges at industrial level due to its low rate of cellulose hydrolysis and most microorganisms employed for ethanol fermentation can not utilize all sugars derived after hydrolysis. To overcome of 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 C_5 and C_6 sugars. Recently Matthew et al. (2005) has found some promising ethanol producing bacteria viz. recombinant *E. coli* K011*, Klebsiella oxytoca*, and *Zymomonas mobilis* for industrial exploitation. SSF process has now improved after including the co-fermentation of multiple sugar substrates present in the hydrolysate. This new variant of SSF is known as simultaneous saccharification and co- fermentation (SSCF) (Wilke et al., 1976; Patel et al., 2005; Wyman et al., 2005). SSF and SSCF are preferred over SHF, since both operations can be performed in the same tank resulting in lower cost, higher ethanol yield and shorter processing time (Wright et al., 1988). The most upgraded form of biomass to ethanol conversion is consolidated bioprocessing (CBP) - featuring cellulose production, cellulose hydrolysis and fermentation in one step-is a highly integrated approach with outstanding potential (Lynd et al., 2005). It has the potential to provide the lowest cost route for biological conversion of cellulo-

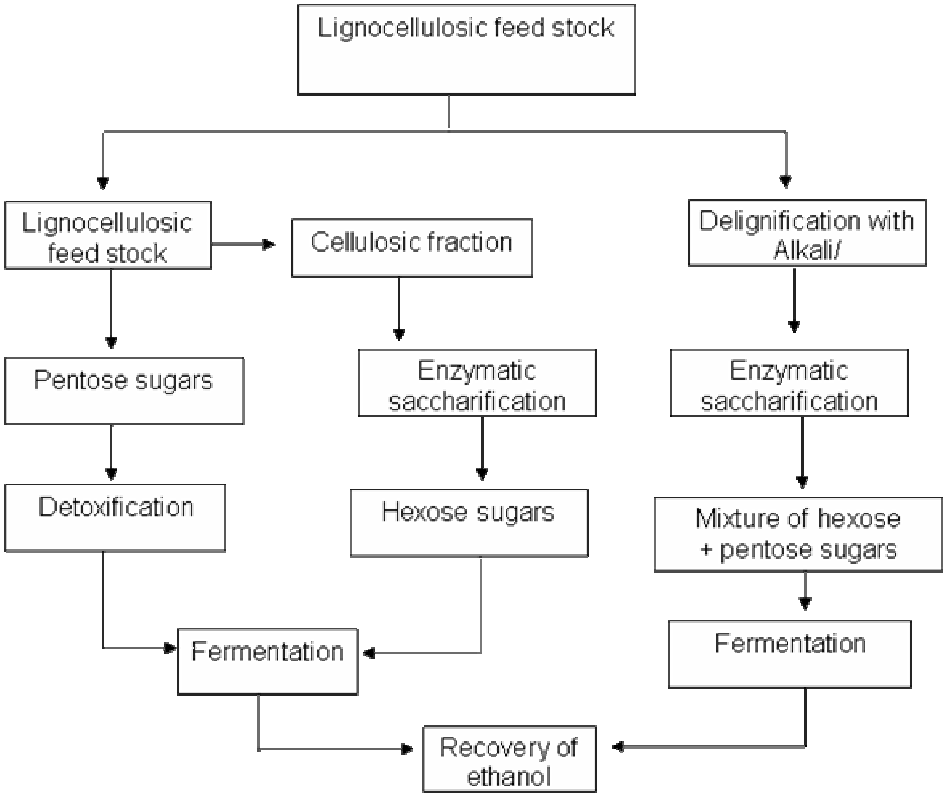

Figure 3. SHF with separate pentose and hexose sugars and combined sugar fermentation.

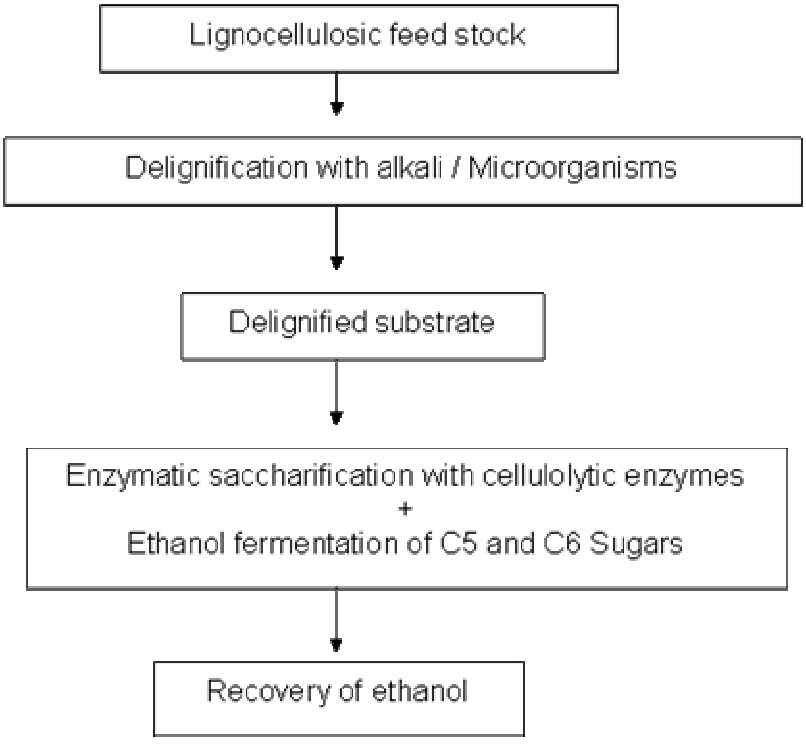

Figure 4. SSF with combined sugars (pentoses and hexoses) fermentation.

losic biomass to ethanol with high rate and desired yields.

Direct microbial conversion (DMC)

DMC is a method of converting cellulosic biomass to ethanol in which both ethanol and all required enzymes 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. Singh and Kumar (1991) found that several strains of *Fusarium oxysporum* have the potential for converting not only D-xylose, but also cellulose to ethanol in a one-step process. 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.

Fermentation

Bioconversion of various biomass sources into ethanol by different microorganisms has been summarized in Table 2. 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 (Toivolla et al., 1984), *S. cereviseae* is capable of converting only hexose sugars to ethanol. The most promising yeasts that have the ability to use both C_5 and C_6 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* (Lin and Tanaka, 2006). 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, Sree et al. (1999) developed solid state fermentation system for ethanol production from sweet sorghum and potato employing a thermotolerant *S. cereviseae* strain (VS3).

Researches are 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 (Jeffries and Jin, 2000; Dien et al., 2003; Katharia et al., 2006). Jeffries and Jin (2004) compiled the recent developments happened towards the genetic engineering of yeast metabolism and concluded that strain selection through mutagenesis, adaptive evolution using quantitative metabolism models may help to further improve their ethanol production rates with increased productivities. Piskur et al., (2006) showed the recent developments in comparative genomics and bioinformatics to elucidate the high ethanol production mechanism from *Saccharomyces* sp.

Though new technologies have greatly improved bioethanol production yet there are still a lot of problems that have to be solved. The major problems include maintaining a stable performance of genetically engineered yeast in commercial scale fermentation operation (Ho et al., 1998, 1999), developing more efficient pre-treatment technologies for lignocellulosic biomass, and integrating optimal component into economic ethanol production system (Dien et al., 2000). Sridhar and co-workers (2002) made an effort to improve the thermo tolerance of yeast isolates by treating them with UV radiation.

Fermentation can be performed as a batch, fed batch or continuous process. The choice of most suitable process will depend upon the kinetic properties of microorganisms and type of lignocellulosic hydrolysate in addition to process economics aspects.

Batch fermentation

Traditionally, ethanol has been produced batch wise. At present, nearly, all of the fermentation ethanol industry uses the batch mode. In batch fermentation, the microorganism works in high substrate concentration initially and a high product concentration finally (Olsson and Han-Hagerdal, 1996). The batch process is a multi-vessel process, allows flexible operation and easy control over the process. Generally batch fermentation is characterized by low productivity with an intensive labour (Shama, 1988). For batch fermentation, elaborate preparatory procedures are needed; and because of the discontinuous start up and shut down operations, high labour costs are incurred. This inherent disadvantage and the low productivity offered by the batch process have led many commercial operators to consider the other fermentation methods.

Fed batch fermentation

In fed batch fermentation the microorganism works at low substrate concentration with an increasing ethanol concentration during the course of fermentation process. Fed batch cultures often provide better yield and productivities than batch cultures for the production of microbial metabolites. For practical reasons, therefore, some continuous operations have been replaced by fed batch process

Table 2. Various raw materials for ethanol production.

Raw Material	Pretreatment and Saccharification	Fermentation conditions	Microorganism	Reference
Sugarcane baggase	Dilute acid hydrolysis	Batch	*C. shehatae* NCIM3501	Chandel et al., 2006b
Wheat straw	Dilute acid, Enzymatic hydrolysis	SSF, SHF	*E. coli FBR5*	Saha et al., 2005
Rice straw	Auto hydrolysis	Batch	*C. shehatae* NCIM3501	Abbi et al., 1996
Sorghum straw	Steam explosion, enzymatic	SSF	*Kluyveromyces marxianus* CECT10875	Ballesteros et al., 2004
Corn stover	Steam, enzymatic	Fed- batch	*S. cereviseae* TMB3400	Ohgren et al., 2006
Barley husk	Steam, enzymatic	SSF	*S. cereviseae*	Palmarola et al, 2005
Sun flower stalk	Steam, enzymatic	Batch	*S. cereviseae var ellipsoideus*	Sharma et al., 2002
Sugarcane leaves	Alkaline H2O2	SSF	*S. cereviseae* NRRL-Y-132	Krishna et al., 2001
Wheat bran	Dilute acid, Enzymatic hydrolysis	Batch	*S. cereviseae*	Palmarola et al, 2005
Ground nut shell	Acid hydrolysis	Batch	*S. cereviseae*	Akpan et al., 2005
Alfalfa fibers	Liquid hot water	SSF, SHF	*C. shehatae* FPL-702	Sreenath et al., 2001
Aspen	Acid hydrolysis	Continuous, Immobilized cells	*P. stipitis* R	Parekh et al., 1987
Saw dust	Acid hydrolysis	Batch, Continuous up-flow reactors	*Clostridium thermosaccharolyticum* ATCC 31925	Liu et al., 1988
Pine	Acid hydrolysis	Continuous stirred tank reactor, Immobilized cells	*P. stipitis* NRRL-1724	Qureshi et al., 1991
Poplar	Steam explosion, Enzymatic	SSF, SHF	*S. cerevisiae*	Cantarella et al., 2004
Birch	Acid hydrolysate	Batch	*S. cerevisiae*	Johanssen et al., 2001
Spruce	Dilute acid hydrolysis	Fed batch	*S. cerevisiae*	Taherzadeh., 1999
Willow	Steam	Batch	*E. coli* K011	Olsson et al., 1995
Paja brava	Dilute acid hydrolysis, Two stage	Batch	*C. shehatae*, *P. stipitis*, *Pachysolen tannophilus*	Sanchez et al., 2004
Eicchornia crassipes	Dilute acid hydrolysis	Batch	*P. stipitis*	Nigam, 2002
Saccharum spontaneum	Dilute acid hydrolysis, Enzymatic	Batch, Fed Batch	*P. stipitis* NCIM 3498	Gupta, 2006
Cassava starch	Starch liquifaction	Batch, Continuous Co-immobilized cells	*S. diastaticus Zymomonas mobilis*	Amutha and Gunashekhran, 2001
Apple pomace		SSF	*S. cerevisiae* ATCC 24702	Ngadi and Correial, 1992
Pine apple canary waste	Juice Extraction	Continuous, Immobilized cells	*S. cereviseae* ATCC 24553	Nigam, 2000
Banana pulp waste	Juice Extraction	Continuous, Cell recycles.	*S. uvarum* NCIM culture 3528	Joshi et al., 2001
Finger Millet (*Eleusine corcana* flour)		High gravity fermentation	*S. cerevisiae*	Reddy and Reddy, 2006
Municipal solid waste (MSW)	Acid pretraetment	Batch	*S. cerevisiae*	Mtui and Nakamura, 2005
News print	Acid hydrolysate	Batch	*E. coli* B (pLOI297)	Lawford and Rousseau, 1993
Industrial waste		SSF	*K. marxianus, S. cereviseae*	Kadar et al., 2004

(Schugerl, 1987). Keeping the low feed rate of substrate solution containing high concentration of fermentation inhibitors such as furfural, hydroxymethyl furfural and phenolics, the inhibitory effect of these compounds to yeast can be reduced. Complete fermentation of an acid hydrolysate of spruce, which was strongly inhibiting in batch fermentation, has been achieved without any detoxification treatment (Taherzadeh, 1999). The productivity

in fed batch fermentation is limited by the feed rate which, in turn, is limited by the cell mass concentration. The specific ethanol productivity has also been reported to decrease with increasing cell mass concentration (Lee and Chang, 1987; Palmqvist et al., 1996). Ideally, the cell density should be kept at a level providing maximum ethanol productivity and yield.

Continuous fermentation

Continuous fermentation can be performed in different kind of bioreactors – stirred tank reactors (single or series) or plug flow reactors. Continuous fermentation often gives a higher productivity than batch fermentation, but at low dilution rates which offers the highest productivities. Alexender et al., (1989) studied the effect of shift in temperature and aeration in steady state continuous culture of *C. shehatae* to determine the effects of ethanol on xylose metabolism. The accu-mulation of ethanol exerted a delayed inhibitory effect on the specific rate of substrate utilization. Continuous operation offers ease of control and is less labor intensive than batch operation. However contamination is more serious in this operation. Since the process must be interrupted, all the equipments must be cleaned, and the operation started again with the growth of new inoculum. The continuous process eliminates much of the unproductive time associated with cleaning, recharging, adjustment of media and sterilization. A high cell density of microbes in the continuous fermenter is locked in the exponential phase, which allows high productivity and overall short processsing of 4 - 6 h as compared to the conventional batch fermentation (24 - 60 h). This results in substantial savings in labour and minimizes investment costs by achieving a given production level with a much smaller plant.

Immobilized cells

A limitation to continuous fermentation is the difficulty of maintaining high cell concentration in the fermenter. The use of immobilized cells circumvents this difficulty. Immobilization by adhesion to a surface (electrostatic or covalent), entrapment in polymeric matrices or retention by membranes has been successful for ethanol production from hexoses (Godia et al., 1987). The applications of immobilized cells have made a significant advance in fuel ethanol production technology. Immobilized cells offer rapid fermentation rates with high productivity - that is, large fermenter volumes of mash put through per day, without risk of cell washout. In continuous fermentation, the direct immobilization of intact cells helps to retain cells during transfer of broth into collecting vessel. Moreover, the loss of intracellular enzyme activity can be kept to a minimum level by avoiding the removal of cells from downstream products (Najafpour, 1990). Immobilization of microbial cells for fermentation has been developed to eliminate inhibition caused by high concentration of substrate and product and also to enhance ethanol productivity and yield. Abbi et al., (1996) observed that the rate of sugar consumption by immobilized cells of *C. shehatae* NCL-3501 was slightly lower than that of free cells, thus leading to higher ethanol production. When microorganisms are attached to solid supports, fluid viscosity is lower which contributes to better mixing and mass transfer in the system. The work on ethanol production in an immobilized cell reactor (ICR) showed that ethanol production using *Z. mobilis* was doubled. Amutha and Gunasekaran (2001) reported ethanol production, 46.7 g/l from 150 g/l liquefied cassava starch from co-immobilized cells of *Saccharomyces diastaticus* and *Z. mobilis*. Yamada et al. (2002) successfully used recombinant *Z. mobilis* with high sugar concentration (12-15%) and further observed the significant role of increased biomass concentration in bioreactor performance for the improved ethanol production. A repeated batch fermentation system was used to produce ethanol using an immobilized osmotolerant *S. cereviseae*, in which ethanol concentration as high as 93 g/l was recorded at 200 g/l glucose concentration (Sree et al., 2000). Nigam (2000) has reported that the ethanol production rate as high as 42.8 g/l/h was achieved from the fermentation of pineapple canary derived sugars by *S. cereviseae* ATCC 24553.

Recycling of process stream

In an environmentally sustainable process, the use of fresh water, the amount of wastewater and the energy consumption must be minimized. The water consumption is decreased by recirculating process streams for use in the washing and hydrolysis steps (Palmqvist and Hahn-Hagerdal, 2000). Recirculating part of the dilute ethanol stream from the fermenter can increase the ethanol concentration in the feed to the distillation stage. However, computer simulations have shown that recirculation of streams leads to the accumulation of non-volatile inhibitory compounds (Galbe and Zacchi, 1992; Palmqvist et al., 1996).

To increase the ethanol productivity, cell recycling has been employed by several workers (Fein et al., 1984; Maleszka et al., 1981), while retaining the simplicity of the batch process. Cell recycling generally does not increase the sugar consumption or ethanol production but the time required for the fermentation can be reduced by 60 - 70%. Schneider (1989) observed a reduction in ethanol production after third cell cycle and suggested the decrease in ethanol production was due to the limitations of oxygen and sugar as a result of an increase in cell density.

Economics of ethanol production technologies

To be competitive, and find economic acceptance, the cost for bioconversion of biomass to liquid fuel must be

lower than the current gasoline prices (Wayman and Parekh, 1990; Subramanian et al., 2005). It seems how-ever; now much more attainable because of increasing efforts of researchers working towards improvisation in the efficiency of biomass conversion technologies (Vertes et al., 2006).

However there is still huge scope to bring down the cost of biomass-to-ethanol conversion. The cost of feedstock and cellulolytic enzymes are two important parameters for low cost ethanol production. Biomass feedstock cost represents around 40% of the ethanol production cost (Hamelinck et al., 2005). An analysis of the potential of bioethanol in short and long term (2030) in terms of performance, key technologies and economic aspects such as cost per kilometer driven has been conducted recently by Hamelinck and Faaij (2006). In this analysis, the production cost of bioethanol was found to be within the range of 16-22 €/GJ_{HHV} (Euro/Giga Joules High Heating Value) at present and down to 13 €/GJ_{HHV} in future (2030). The feedstock cost is major parameter influencing the ethanol production cost at a rate of 2-3 €/GJ_{fuel}. Employing integrated approaches using the larger industrial facilities by integrated action plan along with cheap feedstock and potent cellulases could make the process more economically viable (Sun and Cheng, 2002; Dien et al., 2006)

Securing long supply of energy sources requires not only that existing fuel resources be utilized as economically as possible along with their diversification uses. Keeping this point of view, bioethanol is identified and portrayed as the entity for ensuring energy security in future fuel interests and global requirements.

The choice of feedstock for ethanol production depends upon its availability and the ongoing uses. For example, agroresidues such as wheat straw, sorghum and barley straw are not preferable for bioethanol production due to their use as animal fodder. On the contrary, agroresidues like sugarcane bagasse, rice straw, rice bran, groundnut shell, corn stover, *Brassica carniata* stalks and soyabean stalks etc can be used directly because these sources are not preferably used as fodder for livestock. Some dedicated energy sources like damaged rice and sorghum grains (Suresh et al., 1999), sunflower stalks and hulls (Sharma et al., 2000), *Eicchornia crassipies* (Nigam, 2003), *P. brava* (Sanchez et al., 2004), alfalfa fibers (Sreenath et al., 2001), residual starch and crushed wheat grains (Davis et al., 2006), agro waste (Campo et al., 2006) and *Saccharum spontaneum* (Gupta, 2006) are more feasible sources for bioethanol production. Also, organic waste and municipal solid waste (MSW), which contain significant amount of cellulose could be cost economic friendly and solve the problem of solid waste storage and management.

In India, Department of Biotechnology, Govt. of India funded a nationwide research project towards the use of selective weeds like *Lantana camara, Prosopis juliflora* and fruit or vegetable waste into ethanol conversion due to their vast abundance, low cost and rich in fermentable carbohydrates.

An important factor for reducing the cost of bioethanol production is to use larger industrial facilities rather than smaller ones. Ward and Singh (2002) also suggested the integrated approach (Process engineering, fermentation and enzyme and metabolic engineering) could improve the ethanol production economics. By increasing the plant size, the investment per unit output of product falls off, a ten-fold increase in size reducing the unit cost to less than one-half and thereby reducing unit capital cost charges and conversion cost reducing profitability (Wayman and Parekh, 1990; Henke et al., 2006).

To further improve the economy of ethanol production, energy integration of the ethanol production, to already existing plants such as pulp and paper plants is necessary. O' O'Boyle et al. (1991) reported that the cost of producing ethanol from pine with a diluted acid hydrolysate process, was estimated to be 3.22 SEK L^{-1} in a stand alone plant in comparison to 2.54 SEK L^{-1} with an integrated plant. They projected that the cost of bioethanol can be reduced from US$ 1.22 per liter to about US $ 0.31 per liter on the basis of continuous improvement in pretreatment of biomass, enzyme application and fermentation.

Aristidou and Penttila (2000) reported that the total cost of ethanol will be dropped from more than $1.0 per litre to ~ $0.3-0.5 per litre, with a projected cost of less than $0.25 per litre in the near future.

Wilke et al. (1981) has made the first effort to analyse the cost of the conversion of biomass to ethanol process based on a SHF operation and concluded that neat ethanol could compete with gasoline at the oil prices at $20 to $30 per barrel**.** Foody (1988) has emphasized the importance of improvement in cost effective cellulases production and outlined the potential for bringing down the price of bioethanol from 25-55 cents to 10-28 cents per liter. Subsequently, Wright (1988) and Hinman et al. (1992) evaluated the benefits of SSF process over to SHF technology and reported the cost based on economic evaluation of bioethanol keeping view the SSF mode of operation. Wooly et al. (1999) explained the further economic analysis of bioethanol ($ 0.78 per gallon) and suggested a projected cost of as low as $ 0.20 per liter by 2015 if enzymatic processing and biomass improvement targets are met. The projected cost of ethanol production from cellulosic biomass as per the earlier estimates ($4.63/gallon in 1980) has been reduced by almost a factor of four ($1.22/gallon) over the last 20 years (Wyman, 1999; Wyman, 2001).

However, Kadam et al. (2000) reported that ethanol production cost can be achieved at $ 1.20 per liter by using two-stage dilute acid hydrolysis process. Krishnan et al. (2000) worked out on economics of ethanol production from dry-milled corn starch in fluidized–bed reactors using immobilized *Z. mobilis* cells. They analysed the cost of ethanol for 15 million gal/year production plant

using Aspen Plus (Aspen Technology, Cambridge; MA) process simulation software and concluded that the operating cost savings of ethanol production in the range of 1.1 - 3.1 cents/gallon. Kwaiatkowski and Co-workers (2006) developed a model for cost evaluation of ethanol production from 40 million gal/year ethanol producing facility using corn dry-grind process technology. The model was developed using Super Pro Designer ^{R} software and data collected from ethanol producers, technology suppliers, equipment manufactures and engineers working in the different industries. The cost of ethanol was found to be increased from US$ 0.235/L to US$ 0.365/L as the price of corn increased from US$ 0.071 to US$ 0.125 /kg.

The bioconversion of pentosans and hexosans from lignocellulosic biomass to ethanol need to be achieved with the maximum efficiencies in terms of higher yield and improved productivity to make the biomass-to-ethanol process economical. The economics of the ethanol process is determined by the cost of sugar. The average biomass cost amounts to ~$0.06 per kg of sugar, or a contribution to the feedstock costs for ethanol production of as low as $0.10 per liter (Aristidou and Penttila, 2000).

Wingren et al. (2003) evaluated the SHF and SSF economic using cellulase enzymes in both configurations with SSF being less expensive by about 10%; and estimated the ethanol production cost of 0.56 – 0.67 $/L. Later, Wingren et al., 2004 studied the effect of reduction in yeast and enzyme concentration in SSF process and concluded final ethanol production cost 4.80 SEK/L (2.34 US$/gal). According to the National Renewable Energy Laboratory (NREL, Colarado, USA) estimations, ethanol production cost of 20 cents per litre is possible in another 15 years from lignocellulose biomass employing designer cellulases and SSCF (simultaneous saccharification and co-fermentation) process (Ghose and Ghose, 2003). However, in both the process, the use of cellulase makes the process cost effective (Mielenz, 2001; Alzate et al., 2005; Gray et al., 2006). According to the analysis of US DOE (US Department of energy), if the enzyme cost can be brought for less than 10 cents per gallon of ethanol the cost of making ethanol could drop as low as 75 cents / gallon (Griffith and Atlas, 2005). Apart from focusing the economics of cellulase production cost, several studies are being carried out to improve the ethanol production by improving acid hydrolysis process. Luong and Tseng (1984) evaluated the technoeconomics of ethanol production under continuous culture using immobilized cells of *Z. mobilis* using plug-flow reactor. They found this technology economically attractive and finally concluded that at least 4 cents/gal of ethanol could be saved using immobilized cells rather than the conventional batch system. They further suggested that by switching from batch to immobilized processing; the fixed capital investment is substantially reduced, thus increasing the profitability of ethanol production by fermentation. Von Sivers et al. (1994) analysed the cost economics of ethanol production (48 cents/gallon) from detoxified willow hemicellulosic hydrolysate using recombinant *E. coli* K011. Zacchi and Axelsson (1989) suggested preconcentrating dilute sugar solution using reverse osmosis is economically feasible to getting high ethanol concentration in fermented broth. Cysewki and Wilke (1976) described cell recycle and vacuum fermentation processes for continuous ethanol production on a production capacity of 78,000 gal ethanol / day employing molasses as the fermentation substrate and estimated ethanol production cost 82.3 and 80.6 cent/gal, for the cell recycle and vacuum processes, respectively.

More recently Zhang (2006) analyzed the cost economics of ethanol production from a small-size lignocellulose refinery with a capacity of 100 tones per day producing approximately 3 million gallons of ethanol plus co-products. He estimated the cost of ethanol production ~$1.00-1.20 per gallon.

The distillation cost of per unit amount of ethanol produced is substantially higher at low ethanol concentrations, the researchers have dealt with the idea of concentrating sugar solutions prior to fermentation (Cyweski et al., 1976; Oh et al., 2000; Iraj et al., 2002). Ethanol distillation cost can be further improved by using membrane distillation process. It has the lowest operational cost, flexible, simple to use and is easy to maintain. It is most efficient and cost effective option among the available distillation processes (Gonsalves, 2006).

Ethanol and Environment

Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released carbon dioxide is recycled back into plant material because plants use CO_2 to synthesize cellulose during photosynthesis cycle (Wyman, 1999; Chandel et al., 2006a). Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source (Figure 5). In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources (Wyman and Hinman, 1990). Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect (Foody, 1988).

As energy demand increases the global supply of fossil fuels cause harm to human health and contributes to the green house gas (GHG) emission. Hahn-Hagerdal (2006) alarmed to the society by seeing the security of oil supply and the negative impact of the fossil fuel on the environment, particularly on GHG emissions. The reduction of GHG pollution is the main advantage of utilizing biomass conversion into ethanol (Demirbas, 2007). Ethanol contains 35% oxygen that helps complete combustion of fuel and thus reduces particulate emission that pose health hazard to living beings. A study conducted by Bang-Quan et al. (2003) on the ethanol blended diesel (E10 and E30)

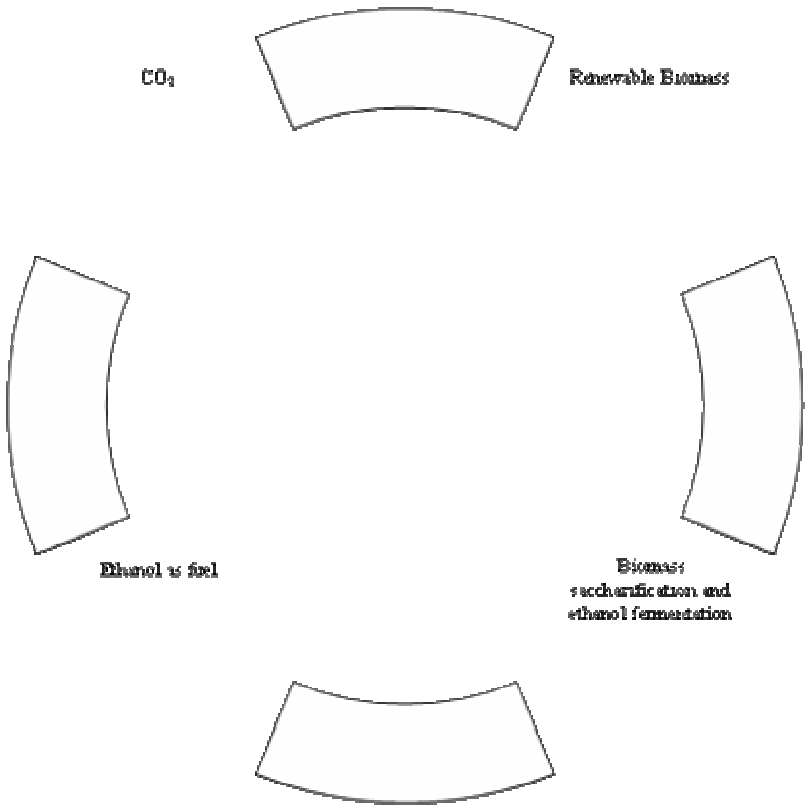

Figure 5. Ethanol represents closed CO_2 cycle.

combustion at different loads found that addition of ethanol to diesel fuel simultaneously decreases cetane number, high heating value, aromatics fractions and kinematic viscosity of ethanol blended diesel fuels and changes distillation temperatures. These factors lead to the complete burning of ethanol and less emissions. With its ability to reduce ozone precursors by 20 - 30%, bioethanol can play a significant role in reducing the harmful gasses in metro cities worldwide. Ethanol blended diesel (E-15) causes the 41% reduction in particulate matter and 5% NO_x emission (Subramanian et al., 2005; Chandel et al., 2006a). One of the disadvantage in using ethanol as fuel is that aldehyde predominantly acetaldehydes emissions are higher than those of gasoline. However acetaldehydes emissions generate less adverse health effects in comparison to formaldehydes emitted from gasoline engines (Gonsalves, 2006).

Environmental impact of bioethanol production technologies and their life-cycle assessment (LCA)

Life-cycle assessment (LCA) is a conceptual framework and methodology for the assessment of environmental impacts of product systems on a cradle-to-grave basis (Graedel, 1999; Tan et al., 2002). Analysis of a system under LCA encompasses the extraction of raw materials and energy resources from the environment, the conversion of these resources into the desired products, the utilization of the product by the consumer, and finally the disposal, reuse, or recycle of the product after its service life (Tan et al., 2002). The LCA approach is an effective way to introduce environmental considerations in process and product design or selection (Azapagic, 1999). Based on life cycle assessment (LCA) studies, ethanol production technologies can be compared. Energy production and utilization cycles based on cellulosic biomass have near-zero green house gas emissions on a life cycle basis (Lynd et al., 1991). Biomass utilization into ethanol production offer environmental benefits in terms of nonrenewable energy consumption and global warming impact. Kim and Dale (2005) studied LCA emphasizing corn and soyabean production and their utilization into bioethanol and biodiesel production and concluded that both the biofuels have environmental benefits in terms of nonrenewable energy consumption and global warming impact. However biomass utilization into ethanol also tends to increase acidification and eutrophication, primarily because large nitrogen, phosphorus are released after cultivation of crops. Lechon et al. (2005) studied the LCA of ethanol production from wheat and barley grain and found that barley is a better option than wheat in terms of fulfillment of the green house gas emissions.

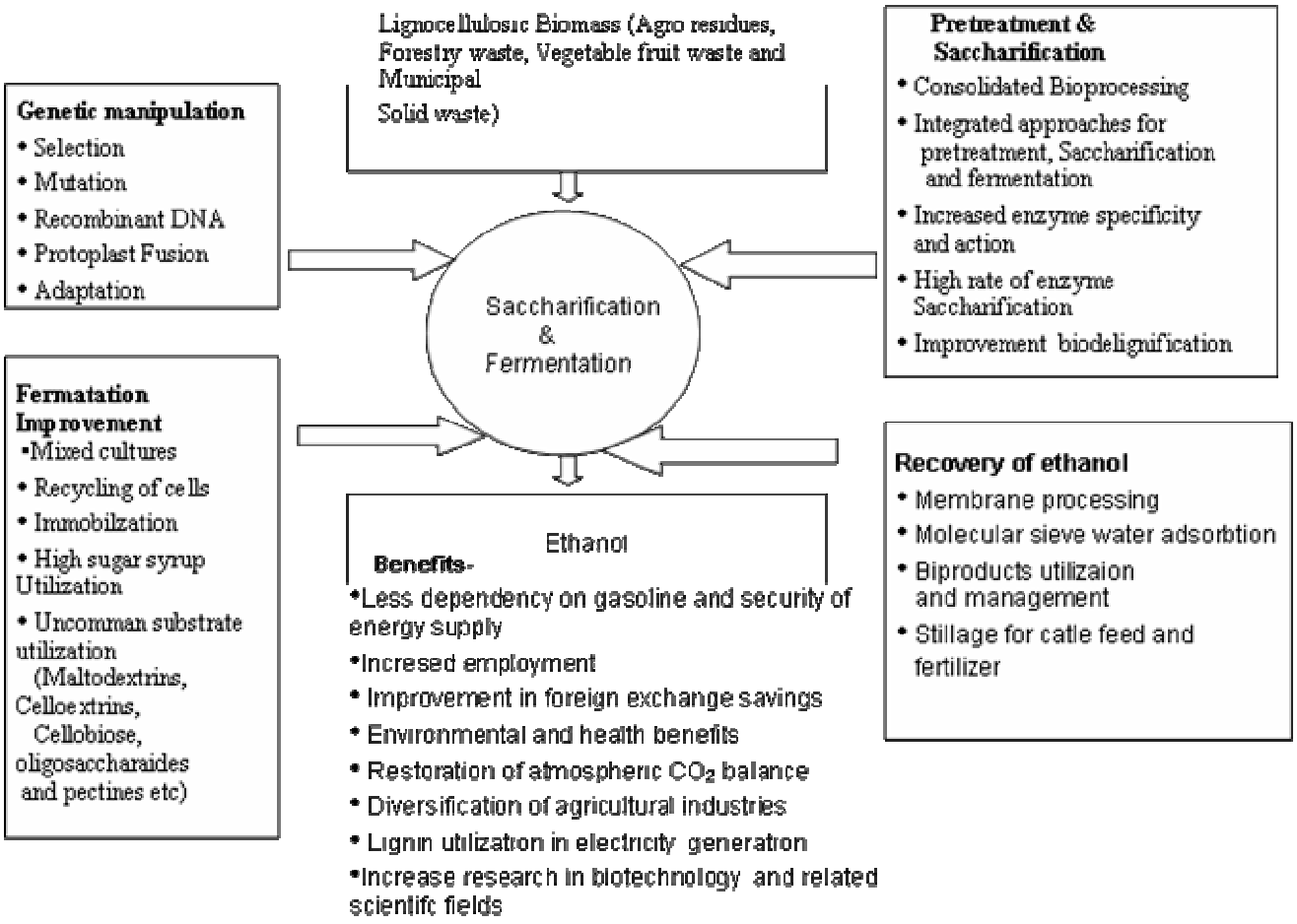

Figure 6. Coordinated action for improvement of biomass to ethanol and its long term benefits.

In an extensive study by Kadam (2000), LCA of acid and enzymatic hydrolysis was compared. All environmental flows were examined from the product life cycle, its production and extraction from raw materials through intermediate conversion process, transportation, distribution and use. Dilute acid process was found better than the enzyme process in terms of greenhouse gas potential, natural resource depletion, acidification potential and eutrophication potential (Kadam, 2002). The reason is dilute acid process sends a much higher proportion of biomass to the boiler for electricity production, which in turn offsets large amounts of emissions (Kadam, 2000). Conversely the concentrated acid process is a net consumer of energy in terms of high acid load and reaction temperature, results in very high values for green house gas (GHG) effect and other impact parameters (Kadam, 1999). Kemppainen and Shonnard (2005) compared the energy consumption and environmental impact for ethanol production using timber wood and recycled news print. The news print conversion into ethanol has a slightly lower overall composite environmental index compared to the timber process. However ethanol production from timber takes less energy, electricity and produces fewer emissions.

A study conducted by Hu et al. (2004) revealed that the E-85 fueled vehicle is better vehicle than the gasoline fueled car by balancing of all the 3E"s the energy, environmental and economic aspects. E-85 fuelled FFV (fossil fuelled vehicle) is about 15% higher efficient when compared to gasoline fuelled car. It also lowers the pollutant emission viz. particulate matter, CO_2, CO, NOx emission than gasoline fuelled car. E-85 fuelled vehicle is higher in total energy consumption and a good combined energy indicator. This was also in agreement with the recent life cycle-based (well to wheel) studies of fuel / propulsion alternatives for light–duty vehicles with a focus on lignocelluloses derived fuel ethanol by reducing 86% lower life cycle green house gas emissions as compared to the gasoline (Fleming et al., 2006).

Tonan et al. (2006) has discussed the integrated assessment of energy conversion processes by evaluating the thermodynamic, economic and environmental parameters and found that water and air emissions of the plant producing ethanol are relatively low. The tansformity of

ethanol (1.32 x 10^5 seJ/J) is quite high if compared to that of fossil fuel (5.4 x 10^4 seJ/J). This is even when bioethanol production is driven by a large amount of non-renewable inputs (fertilizer, fuel, machinery).

Conclusion

In spite of laboratory based bioethanol success stories, the production of fuel ethanol at plant scale still remains a challenging issue. A positive solution to this issue could bring economic advantage not only for fuel and power industry, but also benefit the environmental rehabilitation and balance issues and cause.

Worldwide, there is only one company, Iogen Corporation, Canada (http://www.iogen.ca), produce bio-ethanol at commercial scale using wheat straw and corn stover. In India, despite plentiful availability of biomass, there is no commercial ethanol production plant from lingocelluloses. The key to the establishment of a commercial bioethanol production facility and the reduction in capital thereof, resulting in lessening of operating costs from each of the units of operations will be an achievement par standards of excellence and utility! Industry attention, not just the accolades is required for searching the answers to the fast paced fuel drain phenomena threatening to takeover into as a major crisis or even worse an economic depression by the end of 21st century!

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 and 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, tailored cocktails of enzymes for the cellulose breakdown coupled with the recent development of genetically engineered microorganism those ferment all possible sugars in biomass to ethanol at high productivity are the major key factors to make bioethanol program successful at commercial scale (Figure 6).

The other important aspect by deploying the bioethanol option is its benefit to the environment. Ethanol is one of the best tools to fight vehicular pollution; its clean burning reduces the harmful gasses and particulate emissions that pose health hazard. The implementation of bioethanol policy can be helpful in improving in envi-ronment and rural economic development with sustainable agricultural practices and enhancement of biomass feedstock conscious usage towards the bioethanol industry will bring up the new age farmer into the limelight and horizon of activities and threshold of business to become renewed with options to deal better in life! A better farmer will ultimately usher in a better livelihood for one and all!

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REFERENCE

Abbi M, Kuhad RC, Singh A (1996a). Fermentation of xylose and rice straw hydrolysate to ethanol by *Candida shehatae* NCL-3501. J. Ind. Microbiol. 17: 20-23.

Abbi M, Kuhad RC,Singh A (1996b.) Bioconversion of pentose sugars to ethanol by free and immobilized cells of *Candida shehatae* NCL-3501: Fermentation behaviour. Proc. Biochem.31: 55-560.

Alexender MA, Chapman TW, Jeffries TW (1989). ontinuos- culture responses of *Candida shehatae* to shits in temperature and aeration: Implications for ethanol inhibition. Appl. Environ. Microbiol. 55: 2152-2154.

Amutha R, Gunasekaran P (2001). Production of ethanol from liquefied cassava starch using co-immobilized cells of *Zymomonas mobilis* and *Saccharomyces diastaticus* J. Biosci. Bioeng. 92: 560-564.

Alkasrawi A, Rudolf A, Lid´en G, Zacchi G (2006). Influence of strain and cultivation procedure on the performance of simultaneous saccharification and fermentation of steam pretreated spruce. Enzyme Microb. Technol. 38: 279–286.

Alzate CAC, Sánchez Toro OJ (2005). Energy consumption analyses of integrated flow sheets for production of fuel ethanol from lignocellulosic biomass. Energy (In Press).

Aristidou A, Penttilä M (2000). Metabolic engineering applications to renewable resource utilization. Curr. Opin. Biotechnol. 11: 187–198.

Aro N, Pakula T, Penttiillae M (2005). Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiol. Rev. 29: 719-739.

Banat IM, Nigam P, Singh D, Marchant P and McHale AP (1998) Ethanol production at elevated temperatures and alcohol concentrations. Part I: Yeasts in general. World J Microbiol Biotechnol. 14: 809–821.

Berg C (2004). World Ethanol Production. The Distillery and Bioethanol Network. Available at http://www.distill.com/ world ethanol production.htm.

Bull SR, Riley CJ, Tyson KS and Costella R (1992). Total fuel cycle and emission analysis of biomass to ethanol . In: Energy from Biomass and wastes vol . XVI (Klass DL ed.) Institute of gas technology , Chicago , IL. pp. 1-14.

Bustos G, Ramirez JA, Garrote G, Vazquez M (2003). Modeling of the hydrolysis of sugarcane bagasse with hydrochloric acid. Appl. Biochem. Biotechnol. 104: 51-67.

Cadoche L, Lopez GD (1989). Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. Biol. Wastes 30: 153–157.

Chaudhuri BK, Sahai V (1993). Production of cellulose enzyme from lactose in batch and continuous cultures by a partially constitutive strain of *Trichoderma reesei*. Enzyme Microb. Technol. 15: 513–518.

Cysewski GR, Wilke CR (1976). Utilization of cellulosic materials through enzymatic hydrolysis. I. Fermentation of hydrolysate to ethanol and single cell protein. Biotechnol. Bioeng.18: 1297– 1313.

Damaso MCT, Castro Mde, Castro RM, Andrade MC, Pereira N (2004). Application of xylanase from *Thermomyces lanuginosus* IOC-4145 for enzymatic hydrolysis of corncob and sugarcane bagasse. Appl. Biochem. Biotechnol. 115: 1003-1012.

Dien BS, Nichols NN, O'Bryan PJ, Bothast RJ (2000). Development of new ethanologenic *Escherichia coli* strains for fermentation of lignocellulosic biomass. Appl. Biochem. Biotechnol. 84/86: 181-196.

Dien BS, Cotta MA, Jeffries TW (2003). Bacteria engineered for fuel ethanol production current status. Appl. Microbiol. Biotechnol. 63: 258–266.

Dien BS, Jung HJG, Vogel KP, Casler MD, Lamb JAFS, Iten L, Mitchell RB, Sarath G (2006). Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass and switchgrass. Biomass and Bioenergy In Press

Draude KM, Kurniawan CB, Duff S JB (2001). Effect of oxygen delignification on the rate and extent of enzymatic hydrolysis of lignocellulosic material. Biores. Technol. 79(2): 113-120.

Eggeman T, Elander TR (2005). Process and economic analysis of pretreatment technologies Biores. Technol. 8: 2019-2025.

Fan LT, Gharpuray MM, Lee YH (1987). In: Cellulose Hydrolysis Biotechnology Monographs. Springer, Berlin. p. 57.

Fein JE, Tallim SR, Lawford GR (1984). Evaluation of D-xylose

fermenting yeasts for utilization of a wood-derived hemicellulose hydrolysate. Can. J. Microbiol. 30: 682–690.
Fleming JS, Habibi S, MacLean HL (2006). Investigating the sustainability of lignocellulose-derived fuels for light-duty vehicles. Trans. Res. Part D 11: 146–159.
Foody B (1988). "Ethanol from Biomass: The Factors Affecting It's Commercial Feasibility." Iogen Corporation, Ottawa, Ontario,Canada.
Foreman PK , Brown D , Dankmeyer L, Dean R , Diener S , Dunn-Coleman NS, Goedegebuur F, Houfek TD, England GJ, Kelley AS, Meerman HJ, Mitchell T, Mitchinson C, Olivares HA, Teunissen PJM, Yao J, Ward M (2003). Transcriptional regulation of biomass-degrading enzymes in the filamentous fungus *Trichoderma.* J. Biol. Chem. 278: 31988-31997.
Galbe M, Zacchi G (1992). Simulation of ethanol production processes based on enzymatic hydrolysis of lignocellulosic materials using Aspen. Appl. Biochem. Biotechnol. 34-35: 93-104.
Ghose TK, Bisaria VS (1979). Studies on mechanism of enzymatic hydrolysis of cellulosic substances. Biotechnol Bioeng. 21:131–146.
Goel AK (2002). A sugar industry perspective. Ethanol: Winrock International India, New Delhi, August 2002. p. 5.
Godia F, Casas C, Sola C (1987). A survey of continuous ethanol fermentation systems using immobilized cells. Process Biochem. 22-22: 43–48.
Gray KA, Zhao L and Emptage M (2006). Bioethanol. Curr Opin Chem Biol 10:141-146.
Gregg DJ, Saddler JN (1996) Factors affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process. Biotechnol Bioeng. 51: 375 – 383.
Griffith M, Atlas RM (2005). Preemptive bioremediation: applying biotechnology for clean industrial products and processes. In: R.M. Atlas and J. Philp (eds.). Bioremediation: Applied microbial solutions for real-world environmental cleanup. ASM press, Washington, D.C. pp. 318-356.
Gupta P (2006). Bioconversion of *Saccharum spontaneum* into fuel ethanol by *Pichia stipitis* NCIM3498. M.Sc.Thesis. Department of Microbiology, University of Delhi, South Campus, New Delhi, India.
Hayn M, Klinger R, Esterbauer H (1993). Isolation and partial characterization of a low molecular weight endoglucanase from *Trichoderma reesei*. *Suominen, P.* and *Reinikainen, T*. Proceedings of the second TRICEL symposium on *Trichoderma reesei* cellulases and other hydrolases. Helsinki, Foundation for Biotechnical and Industrial Fermentation Research. pp. 147-151.
Herrera S (2004). Industrial biotechnology- a chance at redemption. Nature Biotechnol. 22:671-675
Hinman ND, Schell DJ, Riley CJ, Bergeron PW, Walter PJ (1992). Preliminary estimate of the cost of ethanol production for SSF technology. Appl. Biochem. Biotechnol. 34/35: 639-649.
Ho NWY, Chen Z, Brainard AP (1998). Genetically engineered *Saccharomyces* yeast capable of effective co-fermentation of glucose and xylose. Appl. Environ. Microbiol. 64: 1852–1859.
Ho NWY, Chen Z, Brainard A, Sedlak M (1999). Successful design and development of genetically engineered *Saccharomyces* yeasts for effective cofermentation of glucose and xylose from cellulosic biomass to fuel ethanol. Adv. Biochem. Eng. Biotechnol. 65: 164-192.
Holtzapple MT, Lundeen JE, Sturgis R (1992). Pretreatment of lignocellulosic municipal solid waste by ammonia by explosion (AFEX). Appl. Biochem. Biotechnol. 34/35: 5–21.
Hu Z, Pu G, Fang F, Wang C (2004). Economics, environment, and energy life cycle assessment of automobiles fueled by bio-ethanol blends in China. Renewable Energy 29: 2183-2192.
International Energy Agency (2001). World Energy outlook 2000. Second edition, France, International energy agency, February 2001, 75775 Paris cedex 16, France.
Iraj N, Giti E, Lila A (2002). Isolation of flocculating Saccharomyces cerevisiae and investigation of its performance in the fermentation of beet molasses to ethanol. Biomass Bioenergy 23: 481–486.
Itoh H, Wada M, Honda Y, Kuwahara M & Watanabe T (2003). Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white-rot fungi. J. Biotechnol. 103 (3): 273-280.
Jeffries TW, Jin YS (2000). Ethanol and thermotolerance in the bioconversion of xylose by yeasts. Adv Appl Microbiol 47: 221–268.
Kadam KL, Camobreco Glazebrook, Forrest LH, Jacobson WA, Simeroth DC, Blackburn W, NKC (1999). Environmental Life cycle
implications of fuel oxygenate production from California biomass. NREL Report no. NREL/TP-580-25688, National Renewable Energy Laboratory Golden, Colorado.
Kadam KL, McMillan JD (2003). Availability of corn stover as a sustainable feedstock for bioethanol production. Bioresour Technol 18: 17–25
Kapoor RK, Chandel AK, Kuhar S, Gupta R, Kuhad RC (2006). Bioethanol from crop residues, production forecasting and economics: An Indian perspective. In: Lignocellulose biotechnology: current and future prospects (Kuhad RC and Singh A eds). I.K.International, New Delhi, India. (In Press).
Keim CR, Venkatasubramanian K (1989). Economics of current biotechnological methods of producing ethanol. Trends Biotechnol. 7: 22-29.
Keller FA, Hamilton JE, Nguyen QA (2003). Microbial pretreatment of biomass: potential for reducing severity of thermochemical biomass pretreatment. Appl. Biochem. Biotechnol. 27-41: 105-108.
Kheshgi HS, Prince RC, Marland G (2000). The potential of biomass fuels in the context of global climate change; Focus on transportation fuels. Annual Rev. Energy Environ. 25: 199-244
Kim HT, Kim JS, Sunwoo C, Lee YY (2003). Pretreatment of corn stover by aqueous ammonia. Biores Technol. 90: 39-47.
Kim S, Dale EB (2004). Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy 26: 361–375.
Kim S, Holtzapple MT (2006). Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresour Technol. 96: 1994-2006.
Klinke HB,Thomsen AB, Ahring BK (2004). Inhibition of ethanol producing yeast and bacteria by degradation products produced during pretreatment of biomass. Appl. Microbiol. Biotechnol. 66:10-26.
Krishna SH, Reddy TJ, Chowdary GV (2001). Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using thermotolerant yeast. Biores. Technol. 77: 193-196.
Kroumov AD, M´odenes AN, de Araujo Tait MC (2006). Development of new unstructured model for simultaneous saccharification and fermentation of starch to ethanol by recombinant strain. Biochem. Eng. J. 28: 243–255.
Kuhad RC, Singh A (1993). Lignocellulose biotechnology: current and future prospects. Crit Rev Biotechnol 13: 151–172.
Kuhad RC, Singh A, Eriksson KE (1997). Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57: 45-125
Larsson S, Palmqvist E, Hahn Hagerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvevrant NO (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of soft wood. Enzyme MicrobTechnol 24: 151-159.
Lee CW, Chang HN (1987). Kinetics of ethanol fermentations in membrane cell recycle fermentors. Biotechnol. Bioeng. 29: 1105 – 1112.
Lin Tanaka (2006). Ethanol fermentation from biomass resources: Current state and prospects. Appl. Microbiol. Biotechnol. 69: 627-642.
Lopez MJ, Nichols NN, Dien BS, Moreno J,Bothast RJ (2004). Isolation of microorganisms for biological detoxification of lignocellulosic hydrolysates. Appl. Microb. Biotechnol. 64: 125-131.
Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002). Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol Rev. 66: 506-577.
Lynd LR and Wang MQ (2004). A product-nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. J. Ind. Ecol. 7: 17-32.
Lynd LR, van Zyl WH, McBride JE, Laser M (2005). Consolidated bioprocessing of cellulosic biomass: an update. Curr. Opin. Biotechnol. 16: 577–583.
Maleszka R, Veliky IA, Schneider H (1981). Enhanced rate of ethanol production from D-xylose using recycled or immobilized cells of *Pachysolen tannophilus.* Bioctehnol. Lett. 3: 415-420.
Mamma D, Christakopoulos P, Koullas D, Kekos D, Macris BJ, Koukios

E (1995). An alternative approach to the bioconversion of sweet sorghum carbohydrates to ethanol. Biomass Bioenergy 8: 99-103.
Martín C, Galbe M, Wahlbom CF, Hägerdal BH, Jönsson LJ (2002). Ethanol production from enzymatic hydrolysates of sugarcane
bagasse using recombinant xylose-utilising *Saccharomyces cerevisiae*. Enz. Microb. Technol. 31: 274-282.
Martinez A, Rodriguez ME, York SW, Preston JF, Ingram LO (2000). Effects of $Ca(OH)_2$ treatments ("overliming") on the composition and toxicity of bagasse hemicellulose hydrolysates. Biotechnol Bioeng. 69: 526-536.
Matthew H, Ashley O, Brian K, Alisa E, Benjamin JS (2005). Wine making 101.
Mohagheghi AW, Dowe NW, Schell DW, Chou YCW, Eddy C (2004). Performance of a newly developed integrant of *Zymomonas mobilis* for ethanol production on corn stover hydrolysate. Biotechnol. Lett. 26: 321-325.
Moiser N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005). Features of promising technologies for pretreatment of lignocellulosic biomass Biores. Technol. 96: 673-686.
Mojovic L, Nikolić S, Rakin M, Vukasinović M (2006). Production of bioethanol from corn meal hydrolyzates. Fuel 85: 1750-1755.
Najafpour GD (1990). Immobilization of microbial cells for the production of organic acids. J Sci Islam Repub Iran 1: 172–176.
Nigam JN (2001). Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. J. Biotechnol. 87: 17-27.
Nigam JN (2002). Bioconversion of water-hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to motor fuel ethanol by xylose–fermenting yeast. J. Biotechnol. 97: 107-116.
Nigam JN (2000) Continuous ethanol production from pineapple cannery waste using immobilized yeast cells. J. Biotechnol. 80: 189-93.
Nilvebrant N, Reimann A, Larsson S, Jonsson LJ (2001). Detoxification of lignocellulose hydrolysates with ion exchange resins. Appl. Biochem. Biotechnol. 91-93: 35-49.
Nyguyen QA, Saddler JN (1991). An integrated model for technical and economic evaluation of an enzymatic biomass conversion process. Biores. Technol. 35: 275-282.
Oh KK, Kim SW, Jeong YS, Hong SI (2000). Bioconversion of cellulose into ethanol by nonisothermal simultaneous saccharification and fermentation. Appl. Biochem. Biotechnol. 89: 15–3.
Olsson L, Hahn-Hägerdal B (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb. Technol. 18: 312-331.
Palmarola-Adrados B, Galbe M, Zacchi G (2005). Pretreatment of barley husk for bioethanol production. J. Chem. Technol. Biotechnol. 80: 85-91.
Palmqvist E, Hahn-Hägerdal B, Galbe M, Zacchi G (1996). The effect of water-soluble inhibitors from steam-pretreated willow on enzymatic hydrolysis and ethanol fermentation. Enzyme Microb. Technol. 19: 470-476.
Palmqvist E, Greg H, Meinander NQ, Hanhaggerdal B (1999). Main and interaction effects of acetic acid, furfural, parahydroxybenzoic acid on growth and ethanol productivity of yeasts . Biotechnol. Bioeng. 63: 46-55
Palmqvist E, Hahn-Hagerdal B (2000). Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification and II:inhibitors and mechanisms of inhibition. Bioresour. Technol. 74: 17–33.
Ravindranath NH, Somashekar HI, Nagaraja MS, Sudha P, Sangeetha G, Bhattacharya SC and Abdul Salam P (2005). Assessment of sustainable non-plantation biomass resources potential for energy in India. 29: 178-190.
Rodrigues RCLB, Felipe MGA, Almeida e silva JB, Vitola M, Gomez PV (2001). The influence of pH, temperature and hydrolysate concentration on the removal of volatile and nonvolatile compounds from sugarcane bagasse hemicellulosic hydrolysate treated with activated charcoal before or after vacuum evaporation. Brazilian J. Chem. Eng. 18: 299-311.
Saha BC, Iten LB, Cotta MA, Wu YV (2005). Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Proc. Biochem. 40: 3693-3700.
Sanchez G, Pilcher L , Roslander C, Modig T, Galbe M, Liden G (2004). Dilute-acid hydrolysis for fermentation of the Bolivian straw material *Paja brava*. Biores. Technol. 93: 249-256.
Sawada T, Nakamura Y, Kobayashi F, Kuwahara M, Watanabe T (1995). Effect of fungal pretreatment and steam explosion pretreatment on enzymatic saccharification of plant biomass. Biotechnol. Bioeng. 48: 719-724.
Schneider HI (1989). Conversion of pentoses to ethanol by yeast and fungi .Crit. Rev. Biotechnol. 9: 1-40.
Schugerl K (1987). Bioreaction Engineering (volume 1), John Wiley
Sharma SK (2000). Saccharifixcation and bioethanol production from sunflower stalks and hull. Ph.D thesis. Department of Microbiology, Punjab Agriculture University, Ludhiana, India.
Sharma SK, Kalra KL, Kocher S (2004). Fermentation of enzymatic hydrolysate of sunflower hulls for ethanol production and its scale-up. Biomass Bioenergy 27: 399-402.
Shama G (1988). Developments in bioreactors for fuel ethanol production. Proc. Biochem. 23: 138-145.
Sheehan J, Aden A, Paustian K, Killian K, Brenner J, Walsh M, Nelson R (2003). Energy and environmental aspects of using corn stover for fuel ethanol. J. Ind. Ecol. 7: 117-146.
Singh A, Kumar PK (1991). Fusarium oxysporum: status in bioethanol production. Crit. Rev. Biotechnol. 11(2): 129-47.
Sree NK, Sridhar M, Suresh K, Rao LV, Pandey A (1999). Ethanol production in solid substrate fermentation using thermotolerant yeast. Proc Biochem 34:115-119.
Sree NK, Sridhar M, Suresh K, Banat IM, Rao LV (2000). Isolation of thermotolerant, smotolerant, flocculating *Saccharomyces cerevisiae* for ethanol production. Biores. Technol. 72: 43-46.
Sridhar M, Sree NK, Rao LV (1999). Utilization of damaged sorghum and rice grains for ethanol production by simultaneous saccharification and fermentation Biores Technol 68:301-304.
Sridhar M, Sree NK, Rao LV (2002). Effect of UV radiation on thermotolerance, ethanol tolerance and osmotolerance of *Saccharomyces cerevisiae* VS_1 and VS_3 strains. Biores. Technol. 83: 199-202.
Sun Y, Cheng J (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review Biores Technol 83: 1-11.
Sun Y and Cheng J (2004). Hydrolysis of lignocellulosic materials for ethanol production: a review. Biores. Technol. 83:1-11.
Subramanian KA, Singal SK, Saxena M, Singhal S (2005). Utilization of liquid biofuels in automotive diesel engines: An Indian perspective. Biomass Bioenergy 29: 65-72.
Taherjadeh M (1999). Ethanol from Lignocellulose: Physiological Effects of Inhibitors and Fermentation Strategies. Ph.D.Thesis. Lund University, Lund, Sweden.
Toivola A, Yarrow D, Van-den-bosch E, Van-dijken JP, Sheffers WA (1984). Alcoholic fermentation of D-xylose by yeasts. Appl. Microb. Biotechnol. 47: 1221-1223.
Toman M, Cazorla M (1998). The Clean Development Mechanism: A Primer," *Weathervane*, Resources for the Future, Washington, D.C. Source:
Tonon SMT, Brown F, Luchi A, Mirandola A, Stoppato, Ulgiati S (2006). An integrated assessment of energy conversion processes by means of thermodynamic, economic and environmental parameters Energy. 31: 149-163.
Tucker MP, Kim KH, Newman MM and Nguyen QA (2003). Effects of temperature and moisture on dilute-acid steam explosion pretreatment of corn stover and cellulase enzyme digestibility. Appl Biochem Biotechnol 10: 105–108.
Vidal PF, Molinier J (1988). Ozonolysis of lignin – improvement of *in vitro* digestibility of poplar sawdust. Biomass 16: 1–17.
von Sivers M, Zacchi G, Olsson L and Hahn-Hägerdal B: Cost analysis of ethanol production from willow using recombinant *Escherichia coli*. Biotechnol Prog 1994. 10: 555-560.
Wahlbom CF, Hahn-Hägerdal B (2002) Furfural, 5-hydroxymethyl furfural, and acetoin act as external electron acceptors during anaerobic fermentation of xylose in recombinant *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 78: 172-178.
Wayman M, Parekh SR (1990) Biotechnology of biomass conversion; Fuels and chemicals from renewable resources. Open University Press Milton Keynes.
Wilke CR, Yang RD, Scamanna AF and Freitas RP (1981) Raw material evaluation and process development studies for conversion of

biomass to sugars and ethanol. Biotechnol. Bioeng. 23: 163–183
Wingren A, Galbe M, Zacchi G (2003). Techno-Economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks. Biotechnol. Prog. 19: 1109-1117.
Wright JD (1988). Ethanol from lignocellulosics: An overview. Energy Prog. 84: 71-80
Wyman CE, Hinman ND (1990). Ethanol. Fundamentals of production from renewable feedstocks and use as transportation fuel. Appl Biochem. Biotechnol. 24/25: 735-75.
Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR and Lee YY (2005) Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. Biores Technol. 96 (18): 2026-2032.
Wooley R, Ruth M, Sheehan J, Ibsen K, Majdeski H, Galvez A (1999). Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis: current and futuristic scenarios. NREL/TP-580-26157, 1999, National Renewable Energy Laboratory, Golden, CO.
Yamada T, Fatigati MA, Zhang M (2002). Performance of immobilized Zymomonas mobilis 31821 (pZB5) on actual hydrolysates produced by Arkenol technology. Appl. Biochem. Biotechnol. 98: 899–907.
Yu ZS, Zang HX (2004). Ethanol fermentation of acid hydrolysed cellulosic pyrolysate with *Saccharomyces cerevisiae*. Biores. Technol. 93: 199-204.
Zheng YZ, Lin HM, Tsao GT (1998). Pretreatment for cellulose hydrolysis by carbon dioxide explosion. Biotechnol. Prog. 14: 890–896.