



Lignocellulosic ethanol in India: Prospects, challenges and feedstock availability

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ABSTRACT

India has a pressing need for renewable transportation fuels and bio-ethanol is considered as one of the most important options. Currently the country mandates a use of 5% ethanol blending in motor gasoline in several states. The ethanol for this is mainly sourced from molasses feedstock, but this is barely sufficient to meet the current demand. Lignocellulosic biomass is the alternative but the availability of this resource is poorly documented. Also the technologies for ethanol production from lignocellulosic biomass are under preliminary stages of development which warrants extensive R&D in this field. The review discusses the current status of molasses based ethanol production in India and its limitations, the state of technologies for second generation ethanol production and the availability of feedstock for bio-ethanol production.

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1. Introduction

With the ever increasing demand for energy and fast depletion of petroleum resources, globally there is an increasing interest in alternative fuels, especially liquid transportation fuels (Wyman, 2007; Lynd et al., 2008). Bio-ethanol from lignocellulosic biomass is one of the important alternatives being considered due to the easy adaptability of this fuel to existing engine and because this is a cleaner fuel with high octane rating than gasoline (Wheals et al., 1999; Grad, 2006). Lignocellulosic biomass is considered as the only foreseeable feasible sustainable resource for renewable fuel; but the lignocellulosic ethanol commercialization is largely limited by the lack of cost effective processing technologies and cost of enzymes (Sukumaran and Pandey, 2009a).

India is a country with a positive outlook towards renewable energy technology and is committed to the use of renewable sources to supplement its energy requirements. The country is one among the few nations to have a separate ministry for renewable energy which address the development of biofuels along with other renewable energy sources. In the year 2003, the Planning Commission of the Government of India brought out an extensive report on the development of biofuels (Planning Commission, 2003) and bio-ethanol and biodiesel were identified as the principal biofuels to be developed for the nation. Elaborate policies for promoting both bio-ethanol and biodiesel were formulated and the time frames for enacting the development of biofuels and

implementation of policies were defined. The blending targets for ethanol and biodiesel in gasoline and petroleum diesel, respectively were proposed as 10% and 20% by 2011–2012 (Planning Commission, 2003) and a 5% ethanol blend in gasoline was made mandatory in 11 states and three union territories of the nation. The government's decision for mandatory blending has created an increased demand for fuel grade ethanol, which though at present can be met by the current production capacity, will exceed it once the law has been implemented nationwide or if the blending ratio is increased. Indian distilleries use molasses as the feedstock for ethanol production and the annual supply of molasses is sufficient only for producing approximately 2.7 billion litres of ethanol. Of this, only a minor share is available for fuel use (AIDA, 2006; Sukumaran and Pandey, 2009b). Also the prices of molasses ethanol can soar once the current government subsidies on sugar are lifted and this would mean that other raw materials such as grains or lignocellulosic biomass has to be used for fuel ethanol production. With a huge population to feed and limited land availability, the nation needs to develop bio-ethanol technologies which use biomass feedstock that does not have food or feed value.

For ethanol production; feedstock availability, its variability and sustainability are the main issues to be addressed. Though the generation of biomass residues in the country from agriculture is considerably large (DES-DAC, 2008; Ravindranath et al., 2005), the actual availability of a major share of these residues for bio-ethanol production is questionable. Thus the selection of feedstock for a future technology for lignocellulosic ethanol itself needs careful planning. Compounding the challenges is the fact that the country lacks mature technologies for ethanol production from lignocellu-

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lousic biomass which is by far the most abundant renewable resource that may be exploited (Lynd et al., 2002; Zhang, 2008). Though biomass itself may be cheap, the costs of its processing are relatively higher. Technologies for biomass to ethanol conversion are also under various stages of development. Various bottlenecks in such technologies include the pre-treatment of biomass, enzymatic saccharification of the pretreated biomass, and fermentation of the hexose and pentose sugars released by hydrolysis and saccharification (Ghosh and Ghose, 2003). Each of these problems requires substantial R&D efforts for improved efficiency and process economics. The review discusses the present status of lignocellulosic ethanol in India, with emphasis on feedstock availability and management. It also presents a brief outlook on future prospects of lignocellulosic ethanol for the nation.

2. Current status of fuel ethanol in India

India has 0.5% of the oil and gas resources of the world but 16% of the world's population with the result that the country depends heavily on oil imports to meet the domestic demand (Sukumaran and Pandey, 2009b). More than 70% of the needs of the country are met from imports of crude oil and natural gas. The demand for motor gasoline has been growing at an average annual rate of ~7% during the last decade (MPNG, 2009) and it shows an increasing trend. The current consumption of petrol for transportation needs (motor gasoline) is estimated at 15.23 billion liters annually (MPNG, 2009). The current share of biofuels in the consumption of transportation fuels is extremely low and is confined mainly to 5% blending of ethanol in gasoline which the government had made mandatory in the states of Andhra Pradesh, Goa, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Tamil Nadu, Uttar Pradesh and Uttaranchal and in the union territories of Daman and Diu, Dadra and Nagar Haveli and Chandigarh. The notification from the Ministry of Petroleum and Natural Gas states that 5% ethanol blending of petrol shall be supplied in identified areas if (a) the indigenous price of ethanol offered for ethanol blended petrol program is comparable to that offered by the indigenous ethanol for other alternative uses; (b) the indigenous delivered price of ethanol offered for the ethanol blended petrol program for a particular location is comparable to the import parity price of petrol at that location; and (c) there is adequate supply of ethanol (MPNG, 2004; Gonsalves, 2006). This means that, though the ethanol blending in gasoline is mandatory in the 16 states and three union territories mentioned above, it is still subjected to availability and market fluctuations and the exact figure of the amount of ethanol that goes into blending is not available (Sukumaran and Pandey, 2009b). Thus, the available ethanol is not sufficient for production of molasses based ethanol to meet the total demand for ethanol for doping the entire gasoline. The estimated demand of ethanol for 5% and 10% blending in 2006–2008 and the projected demand for 2017 based on gasoline demand in the transport sector are given in Table 1.

The 2006-demand for ethanol at 5% gasoline doping levels was 0.64 billion liters while the estimated current demand for 10%

blending of entire gasoline sold in India is about 1.5 billion liters. This demand is projected to be 2.2 billion liters in 2017. Indian alcohol industry is fairly mature with 295 distilleries operational across the country with an installed annual capacity of 3.2 billion liters. The alcohol produced in the country is mainly consumed by the liquor industry with the remaining share going into chemical industry. According to a 2006 estimate, the total annual demand for alcohol in the country excluding fuel applications was about 1.3 billion litres which is about 40% of the installed capacity (AIDA, 2006). However, during the same year, the actual production of ethanol was only 1.69 billion litres which means the surplus availability was only 0.39 billion litres which is not sufficient to meet the fuel ethanol demand if the entire gasoline in the country had to be doped at 5% level. The present situation of molasses ethanol production is also not very different. Of course, with the distilleries working at their installed capacity, more ethanol may be generated and channeled to fuel applications but this is still limited by availability of molasses. The yield of molasses is directly related to sugar cane production and it varies from 4% to 4.5% of the cane crushed (Ghosh and Ghose, 2003). The statistics for sugar cane production by ICES-DAC, 2006 for India shows that the country produces about 10 million metric tons (MMT) of cane annually which translates to a theoretical molasses yield of 13.1 MMT, if the entire cane is assumed to be crushed and 4.5% of the crushed cane is the molasses generation (Table 2).

Based on a statistics by All India Distillers Association for a one year period covering 65 distilleries in different parts of the nation, the average efficiency in production of molasses based ethanol is 85% and the amount of fermentable sugars in molasses is about 42% which yields of ~222 L of ethanol per ton of molasses (AIDA, 2006). If all of the molasses had been converted to ethanol, the annual yield would be only around 2.91 billion liters. Out of this, the estimated surplus amount would be far less than the projected current demand of ~1.5 billion liters (at 10% blending). The availability of surplus ethanol from molasses is thus very limited and the fact remains that India's cane production can barely supplement the current demand of ethanol even at 5% blending, if the entire gasoline consumed in the nation needs to be doped. This being the status of molasses ethanol, the other options for first generation ethanol include starchy biomass like

Table 2
Ethanol production potential from molasses.

| Year | Sugar cane production (MMT) | Molasses yield (MMT) | Estimated ethanol yield (billion litres) |
|---------|-----------------------------|----------------------|--|
| 2001 | 295.96 | 13.32 | 2.96 |
| 2002 | 297.21 | 13.37 | 2.97 |
| 2003 | 287.38 | 12.93 | 2.87 |
| 2004 | 233.86 | 10.52 | 2.34 |
| 2005 | 237.08 | 10.67 | 2.37 |
| 2006 | 281.17 | 12.65 | 2.81 |
| 2007 | 355.52 | 16.00 | 3.55 |
| 2008 | 340.56 | 15.33 | 3.40 |
| Average | 291.09 | 13.10 | 2.91 |

Table 1
Annual demand of gasoline in India and the projected ethanol demand for blending. Source: IEA's Energy Statistics of Non-OECD Countries (IEA, 2009).

| Year | Motor gasoline demand/consumption (MMT) | Motor gasoline demand (billion litres) ^b | Ethanol demand @ 5% blending (billion litres) | Ethanol demand @ 10% blending (billion litres) |
|-------------------|---|---|---|--|
| 2006 | 9.46 | 12.80 | 0.64 | 1.28 |
| 2007 | 10.47 | 14.17 | 0.71 | 1.42 |
| 2008 | 11.31 | 15.30 | 0.77 | 1.53 |
| 2017 ^a | 16.41 | 22.21 | 1.11 | 2.22 |

^a Report of the committee on development of biofuels, 2003 (Planning Commission, 2003).

^b Specific gravity taken as 0.739 for calculation.

grains or tubers. However, in a country like India with the world's second largest population to feed, and with more than 238 million people living below the poverty line (NSS, 2007) sparing food crops for ethanol production is not an option. Nevertheless, the nation with its fast growing economy and a consequential increase in energy demand needs an immediate addressing of renewable alternatives for transportation fuels.

The country had ambitious plans of meeting 20% of its diesel requirements through biodiesel by 2020 (Planning Commission, 2003). Since the nation is short of edible oils, the feedstock proposed was non-edible oils from plants like *Jatropha curcas* and *Pongamia pinnata*. Though there was an initial hype following the 2003 planning commission recommendations for developing *Jatropha* plantations for biodiesel production, nothing significant has yet been materialized and there is no biodiesel available in the market. However, some of the molasses ethanol is still finding its way into gasoline blends. The solution to renewable transportation fuels may not exclusively be “bio-ethanol”, but it will definitely play a significant part. With the possibility of first generation bio-ethanol ruled out due to reasons stated above, the most appropriate bio-ethanol technology for the nation would be to produce it from lignocellulosic biomass.

India has long been in the “lignocellulose–ethanol” research with the pioneering efforts of Biochemical Engineering Research Centre, IIT Delhi. As early as in 1980, the centre had erected a demonstration facility for an integrated bioprocess for production of 50 L bio-ethanol per day. This process utilized rice straw as raw material with an ethanol yield of 230 L/ton and with an estimated cost of \$0.54 per litre (Ghosh and Ghose, 2003). Multiple labs across the country are now engaged in R&D on the various aspects of lignocellulosic ethanol including the setting up of a 50 kg/day capacity pilot plant at the National Institute for Interdisciplinary Science and Technology (NIIST) campus. However, the country still lacks mature technologies for ethanol production from lignocellulosic biomass which is by far the most abundant renewable resource. Though biomass itself is cheap, the cost of its processing are relatively higher. Technologies for biomass processing and conversion are also under preliminary stages of development.

3. Technological challenges for lignocellulosic ethanol

Lignocellulosic biomass consists mainly of a polymer of glucose (a C6 sugar) called cellulose. It occurs in the biomass in bound form along with hemicellulose (made of C5 sugar monomers) and lignin (Wyman, 1996). Typically lignocellulosic biomass contains 35–55% cellulose, 20–30% hemicellulose and 10–25% lignin (Ghosh and Ghose, 2003). Lignocellulosic biomass as feedstock for bio-ethanol presents a different set of challenges compared to the molasses or starch based feedstocks (Lin and Tanaka, 2006; Bai et al., 2008). When either starch or lignocellulose is used as feedstock, the polymeric material is broken down into the component sugars which are then fermented to produce alcohol. The process of hydrolysis of biomass to generate sugars is called saccharification. Biomass saccharification can be brought about by chemical or biological means, the latter employing enzymes capable of breaking down the complex biomass. Both cellulose and hemicellulose can be hydrolyzed to yield fermentable sugars which may be then fermented to ethanol (Wyman, 1996; Hahn-Hägerdal et al., 2006). Lignin cannot be used for ethanol production but can be used for various other applications (Hu, 2002; Lora and Glasser, 2002). At the moment commercial production of ethanol from lignocellulose does not take place in India. However, a lot of industrial related R&D is implemented. The different process sections in ethanol production from lignocellulosics will include the several unit operations as outlined in Berg (2004). The typical process for

lignocellulosic ethanol production consists of biomass pre-treatment and detoxification followed by hydrolysis, alcohol fermentation and product recovery (Margeot et al., 2009). The biomass is treated with either dilute acid or alkali at high temperature which helps to liberate the hemicelluloses sugars or lignin, respectively, and makes the cellulose component susceptible to enzymatic hydrolysis (Percival Zhang et al., 2006; Himmel et al., 2007; Kumar and Wyman, 2009). Enzymatic hydrolysis (saccharification) is performed with a cocktail of enzymes in which the cellulose hydrolyzing enzymes (cellulases) are the major component. The sugar rich hydrolysate is then fermented using yeasts to produce alcohol. The hemicellulose fraction separated in the acid pre-treatment scheme can be fermented using pentose fermenting microbes for alcohol production or used for production of other high value chemicals (Preziosi-Belloy et al., 1997; Manman et al., 2008). The different technologies practiced for ethanol production from lignocellulosics more or less follows the same steps with variations in the method of pre-treatment and type of enzymes and microbes employed, besides the use of different feedstocks. Some of these processes are discussed in van Zee et al. (2003).

Various bottlenecks in such processes include the pre-treatment of biomass, enzymatic saccharification of the pretreated biomass, and fermentation of the hexose and pentose sugars released by the hydrolysis and saccharification. Each of these problems require substantial R&D efforts for improved efficiency and process economics. Major technological challenges involved in the unit operations are briefly outlined in the following discussion and also in more detail in our earlier review (Sukumaran and Pandey, 2009).

Pre-treatment

Lignocellulosic biomass in its natural form is a tough feedstock for hydrolysis due to the crystallinity of cellulose and due to the compact packing of cellulose, hemicelluloses and lignin in the plant material (Himmel et al., 2007). The basic objective of pre-treatment is to make this complex polymer accessible to the action of cellulases which is achieved by removal of either hemicellulose or lignin from the matrix or breaking up of the compact packing of these polymers (Venkatesh et al., 2009; Kumar et al., 2009). A wide range of thermal, mechanical and chemical pre-treatment methods and combinations thereof have been reported for achieving these goals (Mosier et al., 2005; Hendriks and Zeeman, 2009). While high temperature acid treatment can hydrolyze cellulose to yield sugars, this process will result in formation of degradation products like 5-hydroxymethyl furfural which will interfere with microbial fermentation (Mosdale, 2008). On the other hand, the enzymatic process for hydrolysis is more efficient, works under ambient conditions and produces no toxic byproducts. In this case, the objective of pre-treatment is to make the biomass amenable to enzymatic hydrolysis, at the same time maximizing the recovery of hemicellulose sugars. This objective is achieved by removal of the lignin fraction using; in majority of the cases a mild acid or alkali treatment. Mild acid treatment will result in hydrolysis of hemicellulose which is recovered in the liquid fraction while lignin and cellulose is recovered as solid fraction which can then be hydrolyzed using enzyme. In the case of alkali pre-treatment, lignin component is dissolved in alkali and removed in liquid fraction while the hemicellulose and cellulose fractions are recovered together in the solid fraction (Wyman, 1996; Taherzadeh and Karimi, 2008). There are also methods which use solvents, steam or even water at high temperature. The methods used for pre-treatment of biomass are reviewed in Sun and Cheng (2002), Mosier et al. (2005) and Hendriks and Zeeman (2009). The challenges which lie ahead will be to identify the composition of the feedstocks and devise the best pre-treatment strategy which will suit the se-

lected feedstock(s). This will need extensive R&D efforts employing different feedstocks and pre-treatment methods besides working out the economics of each combination.

3.2. Enzymatic hydrolysis of pretreated biomass

Enzymatic hydrolysis of the cellulosic component of pretreated biomass is the key step in lignocellulosic biomass to ethanol technology. The yield of sugars from a pretreated feedstock is largely dependent on the type of cellulases and their activities. These features will largely determine the enzyme loading and duration of hydrolysis which in turn determines the overall process economics (Sukumaran and Pandey, 2009a).

Cellulases are enzymes which hydrolyze the β -1,4-D-glucan linkages in cellulose and produce as primary products glucose, cellobiose and cello-oligosaccharides. These are produced by a number of microorganisms and comprise several different enzyme classifications. Three major types of cellulase enzymes are involved in the hydrolysis of native cellulose namely cellobiohydrolase (CBH), endo- β -1,4-glucanase (EG) and β -glucosidase (Schulein, 1988). Endoglucanases produces nicks in the cellulose polymer exposing reducing and non-reducing ends, cellobiohydrolase (CBH) acts upon these reducing and non-reducing ends to liberate cello-oligosaccharides and cellobiose units, and β -glucosidases cleaves the cellobiose to liberate glucose completing the hydrolysis. The complete cellulase system comprising CBH, EG and BG components thus acts synergistically to convert crystalline cellulose to glucose (Bguin and Aubert, 1994; Henrissat, 1994). In the process of converting biomass to glucose, the final step in cellulase mediated hydrolysis catalyzed by beta-glucosidase is of much importance since the substrate of this enzyme – cellobiose which is generated by the action of cellobiohydrolases is a very potent inhibitor of the CBH and EG enzymes at higher concentrations. Cellobiose can decrease the rate of cellulose hydrolysis by CBH and EG as much as 50% at a concentration of 3 g/L (Hindle et al., 2000). This decrease in hydrolysis rate necessitates the addition of higher levels of cellulase enzymes, which in turn increases the overall process economics. Cellobiose accumulation has been a major problem in enzymatic hydrolysis because the commercially used cellulase producing microbes make very low β -glucosidase compared to the other enzyme activities. The low levels of β -glucosidase can lead to accumulation of cellobiose and increase the amount of cellulase needed by several fold.

For lignocellulosic ethanol production, the most desired attributes of cellulases are a preparation which contains the complete hydrolytic machinery, high specific activity, high rate of turn over with native cellulosic biomass as substrate, thermo stability, decreased susceptibility to enzyme inhibition (by cellobiose and glucose), selective adsorption on cellulose and the ability to withstand shear forces (Ghosh et al., 2003). The enzyme complexes of the fungi like *Trichoderma reesei* used in industrial cellulase production are susceptible to tight regulations by induction-repression mechanisms (Jensen et al., 1997) and the rate limiting enzyme – beta glucosidase (BGL) is subject to product (glucose) inhibition. Inducers of cellulase production are often costly additives in the production medium, but there are also reports on the use of alternatives like generation of inducers making use of the transglucosidase activity of crude BGL enzymes (Allen and Mortensen, 1981). Development of BGLs those are less sensitive to glucose inhibition, improving the stability and thermo-tolerance of enzymes and increasing the resistance to shear are other challenges which need attention. A detailed description on the current status of cellulases and their application in bio-ethanol production can be found in Mathew et al. (2008) and Sukumaran (2008).

For lignocellulose hydrolysis, saccharification via biological (enzymatic) means is considered to be the most promising route;

however, this step is also one of the major contributors to the overall ethanol cost. Techno-economic evaluations also show that the cost of cellulase enzyme is a major contributor to the production costs amounting to some 40–49% of the net production costs (McAlloon et al., 2000; Reith et al., 2002). Earlier sensitivity analyses performed on the costing data indicated that at least a 10-fold reduction in cellulase production costs was needed for the process to become economical (Aden et al., 2002). While US-NREL lead projects in collaboration with enzyme industry leaders like Genencor and Novozymes have been able to achieve this goal, the new enzymes have not yet reached the market. Probably the newly developed enzymes have to undergo more trials for the companies might want to wait for the right time to release them. Indian market does not have dedicated enzyme preparations for biomass hydrolysis and the cost of current cellulase preparations available in the country does not seem to satisfy the requirements of bio-ethanol production. Though the major Indian enzyme manufacturers like MAPS India Pvt. Ltd. and TCS India Pvt. Ltd. have cellulases in their product profile, they may not be the optimal preparations for biomass hydrolysis. This is because currently the major market for cellulase enzymes in the country is the textile industry, and the enzymes produced are mostly tailored to meet the requirement of this industry. The properties needed for enzymes to be used in biomass hydrolysis may be entirely different from that used in textile industry and the latter makes the textile cellulases much more expensive (Ghosh and Ghose, 2003). The current commercial preparations of enzymes are slow acting and are subject to problems of feedback inhibition (Holtzapfel et al., 1990; Xiao et al., 2004). Research in different laboratories in the country had resulted in cellulases from several different organisms and with wide variations in the activity (Dutta et al., 2008; Chandra et al., 2009; Aswathy et al., 2010). There have also been attempts to improve cellulase production of microbes using classical mutation (Adsul et al., 2007; Chandra et al., 2009) as well as for cloning the genes of cellulases (Roy et al., 2005; Nagarajan and Krishnan, 2009). Enzymatic hydrolysis of various feedstocks for ethanol production has also been widely attempted with considerable amount of success (Araujo and D'Souza, 1986; Hari Krishna et al., 2001; Sukumaran et al., 2009; Aswathy et al., 2010). Nevertheless, majority of the studies on biomass ethanol are confined to bench scale processes which still needs to be scaled and evaluated for developing commercial processes for ethanol production from biomass. The major breakthroughs needed in India as elsewhere, are reduction in the cost of producing cellulases, improvements in their activity and physical properties like thermo-tolerance.

3.3. Microbes for ethanol fermentation

Fermentation of the sugars generated from enzymatic hydrolysis of biomass is another important step where a lot of technical advances are needed to make lignocellulosic ethanol technology feasible. What is desired in an ideal organism for biomass-ethanol technology would be a high yield of ethanol, broad substrate utilization range, resistance to inhibitory compounds generated during the course of lignocellulose hydrolysis and ethanol fermentation, ability to withstand high sugar and alcohol concentrations, higher temperatures and lower pH and minimal byproduct formation (Picataggio and Zhang, 1996). Unfortunately, all these features seldom exist together in any wild organism and the need of the industry would be to develop an organism which will at least partially satisfy these requirements (Zhang et al., 2009). The ability to use the hemicellulose component in biomass feedstock is critical for any bio-ethanol project. *Saccharomyces cerevisiae* and *Zymomonas mobilis*, the commonly employed organisms used in alcohol fermentation lacks the ability to ferment hemicellulose derived pentose (C5) sugars. While there are organisms that can ferment C5

sugars (e.g. *Pichia stipitis*, *Pachysolen tannophilus*, *Candida shehatae*) the efficiencies are lower. They also need microaerophilic conditions and are sensitive to inhibitors, higher concentrations of ethanol and lower pH (Hahn-Hägerdal et al., 1994; Chandrakant and Bisaria, 1998). Worldwide, lots of R&D efforts are being directed to engineer organisms for fermenting both hexose (C6) and pentose (C5 sugars) with considerable amount of success. (Ohta et al., 1991; Lawford and Rousseau, 2002; Alper et al., 2006; Shaw et al., 2008; Wisselink et al., 2009). Several of the promising results from these studies have also found their way into ethanol production. Indian efforts in this area are very less and confined to a few laboratories. For examples, there are reports on co-fermentation using hexose and pentose fermenting yeasts (Palnitkar and Lachke, 1990), protoplast fusion to impart pentose utilization ability to yeasts (Pasha et al., 2007) and on engineering *Saccharomyces* for C5 utilization (Madhavan et al., 2009). There is a need to invest in serious research towards developing potent strains capable of fermenting the sugars derived from lignocellulose hydrolysis for any success in the Nation's biomass ethanol programs.

4. Feedstock for bio-ethanol: the availability issue

All plant and plant derived materials has great potential to provide renewable energy for the future. Agro and forest residues are the potent feed-stocks with huge amounts generated annually, but the availability of these for bio-ethanol production has to be looked with caution. India has a large share of cultivable land which had been a key factor in the country's socioeconomic development. India is the seventh largest country in the world and 51% of the land is arable against a global average of 11% which by no means is lesser (Sukumaran and Pandey, 2009b). However, the land to man ratio is not as favorable as many other countries with far lesser land resources creating serious problems in land resource management and resulting in land degradation. Moreover, a significant part of the agro-residues generated is consumed for fodder and other applications resulting in a low amount of crop residues available for fuel production.

An accurate estimate of the nation's biomass availability is non-existent and the only statistics that are available are on agricultural production and of forest coverage. Even when biomass resources are documented, there seems to be discrepancy in data between

different agencies and the statistics are often not complete or transparent. Nevertheless, there is sufficient data on agricultural output and there are studies on the amount of residues generated (Ravindranath et al., 2005). The residue to crop ratios derived from these studies are useful in calculating the amount of residues obtained for each crop. A major limitation here is the lack of statistics on the current usage of these residues which is essential for calculating the surplus amounts of various biomass feedstock(s) that may be available for ethanol production. A proper documentation of the nation's biomass resources and their availability for fuel ethanol production compared to other possible applications can help the policy makers as well as the R&D scientists to take a targeted approach in addressing the challenges, be it political, economical or technological. NIIST had recently employed India Market Research Bureau (IMRB international) for a nationwide survey funded by the Technology Information, Forecasting and Assessment Council (TIFAC), Government of India, on the generation and availability of various biomass residues which presented several interesting observations (Pandey et al., 2009). According to the survey, the major agro-residues in terms of volumes generated (in million metric tons – MMT) are rice straw (133.8), rice husk, (22.4) wheat straw (109.9), sugar cane tops (97.8) and bagasse (101.3) (Fig. 1). These account for almost 80% of the residue generated by the crops which were studied.

The statistics for crop residue generation calculated from the Department of Agriculture-crop production data (DES-DAC, 2008) based on conversion factors taken from Ravindranath et al. (2005) also gives similar figures. Looking at the estimated amounts of residues, it is apparent that the most potent feedstocks in terms of generating large quantities are rice straw, sugar cane tops and rice husk and wheat straw, followed by other cereal residues. There are a number of other crop residues like that from cotton and chilli cultivation (18.9 and 0.6 MMT, respectively) Residues from processing of forest products like bamboo and reed may also serve as potent feedstock. Pine needles emerged as an unexpected feedstock from the survey with an estimated annual availability of 1.6 MMT, but the resource presents a problem with respect to collection and logistics. The physical properties, content of cellulose and fermentable pentosans in each of these residues are different and accordingly the processing technologies might have to differ slightly if they are to be used as raw material for ethanol production. Though these resources together might be more than sufficient for satisfy-

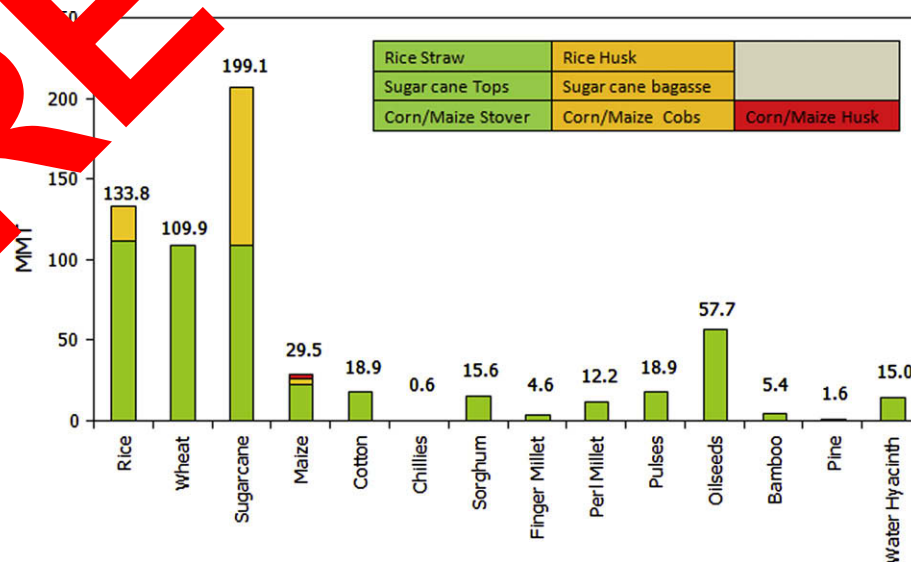


Fig. 1. Annual crop residue generation in India.

ing the Nation's ethanol demand, it is to be noted that the entire residue generated is not available for bio-ethanol production. Part of the residue is consumed as fodder and manure at the source of generation, part by the paper industry, and a part again by its use as a fuel directly by burning. (Pandey et al., 2009). The percentage of residues consumed for these and other unaccounted applications especially in the rural context (thatching, manure, direct burning, etc.) are not available and this points to the need for appropriate and transparent statistics on these. The NIIST-TIFAC survey had tried to address this issue and had done a study on the surplus availability of biomass residues in India. According to the study, sugarcane tops is the most surplus residue (79.4 MMT) as it is almost always burnt in the field itself. Sugar cane bagasse, though generated in good quantities is consumed by free market industries like paper. In such a scenario, biofuel application has to compete with these industries to procure bagasse. Other crops like cotton, chilli, pulses and oilseeds generate considerable amount of surplus biomass because it does not have much use other than fuel. Bamboo processing waste is a major resource to be reckoned considering the 3.3 MMT annual surplus availability of this feedstock. Apart from the processing waste, an additional 4 MMT annual availability of whole bamboo plants from forest is projected by the study. There is very little surplus from cereal crops (rice straw [8.5 MMT], wheat straw [9.1 MMT] and other cereal straws [~6 MMT total]) as it is in much shortage since a major share of it goes for feeding cattle. The major findings of this study are summarized in Fig. 2.

Based on the surplus availability of biomass feedstock, the potential for bio-ethanol generation was calculated for the crop residues – rice straw, wheat straw, sugar cane bagasse and corn stover for which at least the collection systems exists. The results given in Table 3 indicates the potential for generating a total of 5.42 billion liters of ethanol assuming 50% overall efficiency for conversion. This is more than double the projected demand of 2.5 billion liters for gasoline doping at 10% level for 2017.

It may also be noted that this calculation did not include other cereal residues, sugar cane tops, cotton and oilseed straws and bamboo processing waste of which considerable quantities were found to be available in surplus. The theoretical yields of ethanol from these feedstocks are not available. Utilization of these would definitely add onto the alcohol production potential. The procurement price of biomass feedstock is another major concern and for any techno-economic analyses, the cost of feedstock has to be available. The survey has been successful in generating data on the current selling price of various agro-residues and estimating the most likely pricing which might for continuous supply (Table 4).

Table 3

Ethanol production potential from major agro-residues available in surplus.

| Feedstock | Annual availability (MMT) | Theoretical yield ^a (L/ Dry ton) | Max production potential (billion litres) | Max production potential assuming 50% efficiency |
|-------------|---------------------------|---|---|--|
| Rice straw | 8.9 | 416 | 3.70 | 1.85 |
| Wheat straw | 9.1 | 432 | 3.93 | 1.97 |
| Bagasse | 6.4 | 428 | 2.74 | 1.37 |
| Corn stover | 1.1 | 422 | 0.46 | 0.23 |
| Total | | | 10.83 | 5.42 |

^a Theoretical ethanol yield calculated for the conversion factors of US Department of Energy (USDE, 2009).

Nevertheless, these figures are to be viewed with caution since there is a considerable amount of price fluctuation depending on geographical region, local demand and annual crop yield, the latter dependent on climate and rainfall.

Apart from the issue of biomass availability, other major problems to be considered are sustainability and logistics. Indian farming systems are mostly distributed with a huge fraction of the agriculture dependent on rainwater for irrigation (~60% according to Ministry of Agriculture statistics (DES-DAC, 2008)). This creates problems in sustainability and the future bio-ethanol plants have to face the problem of continuous supply of raw materials. One possible solution would be to use multiple feedstocks based on seasonal availability. Since the farming and consequently residue generation is concentrated in distributed pockets, collection and transportation of the feedstocks to the production plants will face certain limitations. Given this scenario, it is prudent to have certain 'anchor suppliers' of biomass for any biofuel manufacturing facility. These anchor suppliers could be existing concentrated sources of biomass like sugar mills and rice mills. For other types of crop residues, co-operatives or other local bodies could be encouraged to collect and supply fixed amount of crop residues over a sustained period similar to the way milk is collected by large cooperatives in many states of India. Either the central or state governments or private parties have to invest on developing infrastructure for biomass collection, processing and selling. Also, the future bio-ethanol plants have to be located in areas where the density of biomass generation and availability are higher. The NIIST-TIFAC survey has identified the states of Uttar Pradesh, Punjab, Tamil Nadu, Haryana, West Bengal and Maharashtra as potent locations with the highest availability of biomass per unit area.

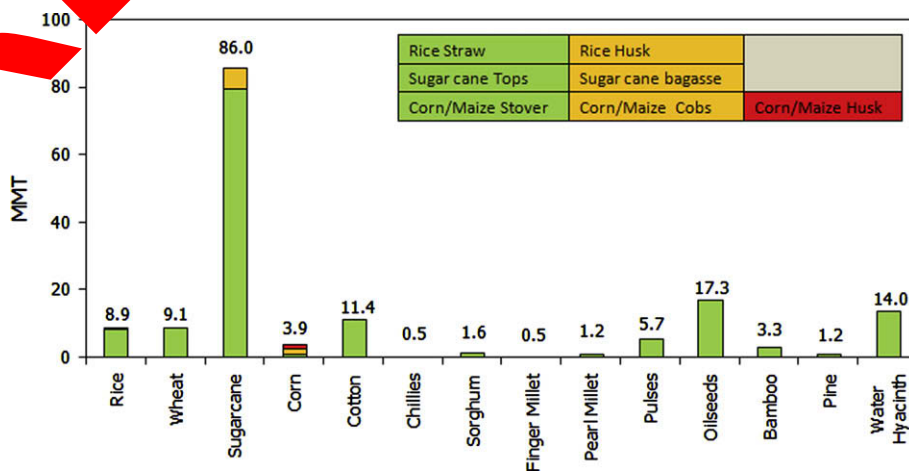
**Fig. 2.** Annual surplus availability of crop residues in India.

Table 4

Procurement prices for major agro-residues in India. Source: NIIST-TIFAC survey (Pandey et al., 2009).

| Crop | Residue | Basic material cost (Rs/Ton) | Likely price (Rs/Ton) |
|--------------------|---------|------------------------------|-----------------------|
| Rice | Straw | 600–1500 | 700 |
| | Husk | 1500–4000 | 1700 |
| Wheat | Straw | 2000–2700 | 2500 |
| | Bagasse | 1350–1500 | 1500 |
| Sugarcane | Tops | Not sold often | – |
| | Stover | 800–1500 | 1000 |
| Maize/corn | Husk | Not sold often | – |
| Cotton | Stalk | 500–800 | 600 |
| Chilli | Stalk | Not sold often | – |
| Jowar/sorghum | Stover | 3000–5000 | 4000 |
| Ragi/finger millet | Stalk | Not sold often | – |

5. Conclusions and perspectives

India is a fast growing economy with an inherent increase in demand for energy. With a huge population and limited land resources, the nation is looking for alternative renewable fuels to support the pace of growth. The demand for liquid transportation fuels is constantly increasing and bio-ethanol might be one of the most potent solutions to the problem. India is one of the largest producers of ethanol and currently all commercial ethanol production in the country uses molasses as feedstock. However, most of it is consumed for application in liquor and chemical industries and the surplus availability can barely support the current demand. A mandatory 5% blending of ethanol in gasoline implemented in several states. This would mean that the demand for ethanol will exceed the surplus production once the law is implemented nationwide or if the blending ratio is increased, which the government is already planning to do. Consequently, sourcing of ethanol from renewable feedstock resources other than molasses is imperative for meeting this increased demand. Lignocellulosic biomass is the only resource that can be used since India does not have surplus grains or other starch biomass to use for fuel applications. However, the technology for ethanol production from lignocellulosic biomass is complex and hence challenging. The country lacks mature technologies for ethanol production from lignocellulosic biomass and though biomass itself is cheap, the costs of its processing are relatively high. Technologies for biomass to ethanol conversion are at the preliminary stages of development. Various bottle necks in such technologies include the pretreatment of biomass, enzymatic saccharification of the pretreated biomass, and fermentation of the glucose and pentose sugars released by hydrolysis and saccharification. Each of these problems require substantial efforts for improved efficiency and process economy. Major initiatives are needed in overall process integration and working out the process scenario, cost inputs and potential gains weighed against it.

One of the major difficulties that would be faced by bio-ethanol technology developers as well as future entrepreneurs will be the choice of feedstock. Though India generates a huge amount of biomass residues as agro- and forest residues, the only feasible feedstock among these would be the crop residues due to problems in collection and logistics. Even in the case of crop residues, the availability is limited due to the use of a major fraction of it as feed and fuel in rural areas. The residues from major agricultural crops like rice wheat and sugar cane are mostly consumed in as fodder or as raw material for competing industries like paper, and less than 10% are available in surplus. A recent nationwide survey on the availability of agro and other biomass residues by NIIST and TIFAC has identified several feedstocks with surplus availability and

therefore the potential to be exploited for bio-ethanol production. However, it would be safer for the future bio-ethanol plants to use multiple feedstocks due to the uncertainty in availability of a given type of residue in sufficient quantities throughout the year. This uncertainty is resultant of the significant dependence of Indian agriculture on rainwater for irrigation, and due to a distributed occurrence of the sources which creates collection and transportation problems. State or private owned initiatives are needed for biomass collection and supply and this single factor will be a significant determinant of the success of any biomass-ethanol technology in India.

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References

- Aden, P.M., Ibsen, K., Janda, R., Nieves, K., Sheehan, J., Wallace, B., et al., 2002. Lignocellulosic Biomass to Ethanol Process Utilizing Auto-current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis for Corn Stover. NREL Technical Report. NREL/TP-510-32438.
- Adst, G., Bastawde, S., Varma, A.J., Gokhale, D.V., 2007. Strain improvement of *Penicillium janthinellum* NCIM 1171 for increased cellulase production. *Bioresour. Technol.* 98 (7), 1467–1473.
- AIDA, 2006. India Distillers Association. <www.aidaindia.org> (accessed 19.05.06).
- Alper, H., Mortensen, R.E., 1981. Production of cellulase from *Trichoderma reesei* in solid state fermentation from soluble carbon sources. *Biotechnol. Bioeng.* 23 (11), 2641–2645.
- Alper, H., Moxley, J., Nevoigt, E., Fink, G.R., Stephanopoulos, G., 2006. Engineering yeast transcription machinery for improved ethanol tolerance and production. *Science* 314, 1565–1568.
- Araujo, A., D'Souza, J., 1986. Enzymatic saccharification of pretreated rice straw and biomass production. *Biotechnol. Bioeng.* 28 (10), 1503–1509.
- Aswathy, U.S., Sukumaran, R.K., Devi, G.L., Rajasree, K.P., Singhania, R.R., Pandey, A., 2010. Bio-ethanol from water hyacinth biomass: An evaluation of enzymatic saccharification strategy. *Bioresour. Technol.* 101 (3), 925–930.
- Bai, F.W., Anderson, W.A., Moo-Young, M., 2008. Ethanol fermentation technologies from sugar and starch feedstocks. *Biotechnol. Adv.* 26 (1), 89–105.
- Berg, C., 2004. World Fuel Ethanol-Analysis and Outlook. <<http://www.distill.com/World-Fuel-Ethanol-A&O-2004.html>> (accessed 19.05.06).
- Bguin, P., Aubert, J.P., 1994. The biological degradation of cellulose. *FEMS Microbiol. Rev.* 13, 25–58.
- Chandra, M., Kalra, A., Sangwan, N.S., Gaurav, S.S., Darokar, M.P., Sangwan, R.S., 2009. Development of a mutant of *Trichoderma citrinoviride* for enhanced production of cellulases. *Bioresour. Technol.* 100 (4), 1659–1662.
- Chandrakant, P., Bisaria, V.S., 1998. Simultaneous bioconversion of cellulose and hemicellulose to ethanol. *Crit. Rev. Biotechnol.* 18, 295–331.
- DES-DAC, 2008. Agricultural Statistics at a Glance, Directorate of Economics and Statistics. Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India. <http://dacnet.nic.in/eands/latest_2006.htm>.
- Dutta, T., Sahoo, R., Sengupta, R., Ray, S.S., Bhattacharjee, A., Ghosh, S., 2008. Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: production and characterization. *J. Ind. Microbiol. Biotechnol.* 35 (4), 275–282.
- Ghosh, P., Ghose, T.K., 2003. Bioethanol in India: Recent Past and Emerging Future. *Adv. Biochem. Eng. Biotechnol.* 85, 1–27.
- Gonsalves, J.H., 2006. An Assessment if the Biofuels industry in India: A United Nations Conference on Trade and Conference. UNCTAD/DIT/TED/2006/6.
- Grad, P., 2006. Biofueling Brazil: An overview of the bioethanol success story in Brazil. *Biofuels* 7 (3), 56–59.
- Hahn-Hägerdal, B., Galbea, M., Gorwa-Grauslund, M.F., Lidén, G., Zacchi, G., 2006. Bio-ethanol: the fuel of tomorrow from the residues of today. *Trends Biotechnol.* 24 (12), 549–556.
- Hahn-Hägerdal, B., Jeppsson, H., Skoog, K., Prior, B.A., 1994. Biochemistry and physiology of xylose fermentation by yeasts. *Enz. Microb. Technol.* 16, 933–943.
- Hari Krishna, S., Janardhan Reddy, T., Chowdary, G.V., 2001. Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast. *Bioresour. Technol.* 77 (2), 193–196.
- Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* 100 (1), 10–18.
- Henrissat, B., 1994. Cellulases and their interaction with cellulose. *Cellulose*, vol. 1. Chapman Hall, London, pp. 169–196.

- Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* 315, 804–807.
- Holtzapfel, M., Cognata, M., Shu, Y., Hendrickson, C., 1990. Inhibition of *Trichoderma reesei* cellulase by sugars and solvents. *Biotechnol. Bioeng.* 36 (3), 275–287.
- Hu, T.Q., 2002. In Hu, T. Q (ed) Chemical Modification Properties, and Usage of Lignin, Kluwer Academic/Plenum. New York.
- IEA., 2009. India Statistics, International Energy Agency www.iea.org/Textbase/stats/countryresults.asp?COUNTRY_CODE=IN&Submit=Submit.
- Ilmen, M., Saloheimo, A., Onnela, M.L., Penttilä, M.E., 1997. Regulation of cellulase gene expression in the filamentous fungus *Trichoderma reesei*. *Appl. Environ. Microbiol.* 63, 1298–1306.
- Kumar, R., Mago, G., Balan, V., Wyman, C.E., 2009. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresour. Technol.* 100, 3948–3962.
- Kumar, R., Wyman, C.E., 2009. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnol. Prog.* 25, 302–314.
- Lawford, H.G., Rousseau, J.D., 2002. Performance testing of *Zymomonas mobilis* metabolically engineered for cofermentation of glucose, xylose, and arabinose. *Appl. Biochem. Biotechnol.* 98–100, 429–448.
- Lin, Y., Tanaka, S., 2006. Ethanol fermentation from biomass resources: Current state and prospects. *Appl. Microbiol. Biotechnol.* 69 (6), 627–642.
- Lora, J.H., Glasser, W.G., 2002. Recent Industrial Applications of Lignin: A Sustainable Alternative to Nonrenewable Materials. *J. Polym. Environ.* 10 (1–2), 39–48.
- Lynd, L.R., Laser, M.S., Bransby, D., Dale, B.E., Davison, B., Hamilton, R., Himmel, M., Keller, M., McMillan, J.D., Sheehan, J., Wyman, C.E., 2008. How biotech can transform biofuels. *Nat. Biotechnol.* 26, 169–172.
- Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.* 66, 506–577.
- Madhavan, A., Tamalampudi, S., Ushida, K., Kanai, D., Katahira, S., Srivastava, A., Fukuda, H., Bisaria, V.S., Kondo, A., 2009. Xylose isomerase from polycentric fungus *Orpinomyces*: gene sequencing, cloning, and expression in *Saccharomyces cerevisiae* for bioconversion of xylose to ethanol. *Appl. Microbiol. Biotechnol.* 82 (6), 1067–1078.
- Mamman, A.S., Lee, J.M., Kim, Y.C., Hwang, I.T., Park, N.J., Hwang, Y.K., Chang, J.S., Hwang, J.S., 2008. Furfural: Hemicellulose/xylose derived biochemical. *Bioresour. Technol.* 99 (5), 438–454.
- Margeot, A., Hahn-Hagerdal, B., Edlund, M., Slade, R., Monot, F., 2009. New improvements for lignocellulosic ethanol. *Curr. Opin. Biotech.* 20 (3), 372–380.
- Mathew, G.M., Sukumaran, R.K., Singhania, R.R., Pandey, A., 2008. Progress in research on fungal cellulases for lignocellulose degradation. *J. Ind. Res.* 898–907.
- McAloon, A., Taylor, F., Yee, W., Ibsen, K., Wooley, R., 2001. Determining the cost of producing ethanol from corn starch and lignocellulose feed stocks. *NREL Report* 580, 28893.
- MPNG, 2009. Petroleum Statistics. Ministry of Petroleum and Natural Gas, Government of India. <http://petroleum.nic.in/petstat.pdf>.
- Mosdale, D.M., 2008. In: Mosdale, D.M. (Ed.) Biofuels: Biotechnology, Chemistry and Sustainable Development. CRC Press and Francis, Boca Raton, FL.
- Mosier, N., Wyman, C., Dale, B., Elander, P., Lee, M., Holtzapfel, M., Ladisch, 2005. Features of promising technologies for pretreatment. *Bioresour. Technol.* 96, 673–686.
- MPNG, 2004. Ministry of Petroleum and Natural Gas, Government of India. <http://petroleum.nic.in/gazette> (notification 24.10.04).
- Nagarajan, D.R., Krishnan, 2009. Isolation of a new catabolite repression resistant promoter isolated from *Escherichia coli* K12 for hyper-production of recombinant enzymes. *Protein Expr. Purif.* 10.1016/j.pep.2009.09.020.
- NSS, 2007. National Sample Survey Reports, National Sample Survey Organization. Ministry of Statistics and Programme Implementation, Government of India. http://mospi.nic.in/mospi/reports/rept_pubn.htm.
- Ohta, K., Nakamura, D.S., Naga, I.P., Shanningsham, K.T., Ingram, L.O., 1991. Genetic improvement of *Zymomonas mobilis* for ethanol production: chromosomal integration of *Zymomonas mobilis* genes encoding pyruvate decarboxylase and alcohol dehydrogenase II. *Appl. Environ. Microbiol.* 57, 893–900.
- Palnitkar, S.S., Lallapada, A.H., 1990. Efficient simultaneous saccharification and fermentation of agricultural residues by *Saccharomyces cerevisiae* and *Candida shehatae*-the D-xylose fermenting yeast. *Appl. Biochem. Biotechnol.* 26 (2), 151–158.
- Pandey, A., Biswas, S., Sukumaran, R.K., Kaushik, N., 2009. Study on Availability of Indian Biomass Resources for Exploitation: A Report Based on a Nationwide Survey. TIFAC, New Delhi.
- Pasha, C., Kuhad, R.C., Rao, L.V., 2007. Strain improvement of thermotolerant *Saccharomyces cerevisiae* VS strain for better utilization of lignocellulosic substrates. *J. Appl. Microbiol.* 103 (5), 1480–1489.
- Percival Zhang, Y.H., Himmel, M.E., Mielenz, J.R., 2006. Outlook for cellulase improvement: screening and selection strategies. *Biotechnol. Adv.* 24, 452–481.
- Piccataggio, S., Zhang, M., 1996. Microorganism development for bioethanol production from hydrolysates. In: Wyman, C.E. (Ed.), Handbook on Bioethanol: Production and Utilization. Taylor and Francis, Washington, DC, pp. 163–178.
- Planning Commission, 2003. Report of the Committee on Development of Biofuels. Planning Commission, Government of India. http://planningcommission.nic.in/reports/genrep/cmtt_bio.pdf.
- Preziosi-Belloy, P., Nollet, V., Navarro, J.M., 1997. Fermentation of hemicellulosic sugars and sugar mixtures to xylitol by *Candida parapsilosis*. *Enz. Microb. Technol.* 21 (2), 124–129.
- Ravindranath, N.H., Somashekar, H.I., Nagaraja, M.S., Sudha, P., Sangeetha, G., Bhattacharya, S.C., Abdul Salam, P., 2005. Assessment of sustainable non-plantation biomass resources potential for energy in India. *Biomass. Bioenergy.* 29 (3), 178–190.
- Reith, J.H., den Uil, H., van Veen, H., de Laat, W., Nijssen, J.J., de Jong, E., Elbersen, H.W., Weusthuis, R., van Dijk, H., van den Broek, J., 2002. Co-production of bioethanol, electricity and heat from biomass residues. In: 12th European Conference and Technology Exhibition on Biomass from Energy, Industry and Climate Protection 17–19 June 2002, Amsterdam, The Netherlands.
- Roy, P., Mishra, S., Chaudhuri, 2005. Characterization, sequence analysis, and characterization of a novel α -glucosidase-like enzyme from *Pichia etchellsii*. *Biochem. Biophys. Res. Commun.* 336 (2), 299–303.
- Schulein, M., 1988. Cellulases of *Trichoderma reesei*. In: Wood, W.A., Abelson, J.N. (Eds.), Methods in Enzymology 160. Academic Press, New York, pp. 234–242.
- Shaw, A.J., Podkany, K.K., Desai, R., Bailey, J.S., Rogers, S.R., Thorne, P.G., Hogsett, D., Lynd, L.R., 2008. Genetic engineering of a thermophilic bacterium for production of ethanol at high yield. *Proc. Natl. Acad. Sci. USA* 105, 13769–13774.
- Sukumaran, R.K., 2008. Ethanol from lignocellulosic Biomass: Part II – Production of cellulases and hemicellulases. In: Pandey, A. (Ed.), Handbook of Biomass Based Biofuels. CRC Press, Florida, USA, pp. 141–157.
- Sukumaran, R.K., Pandey, A., 2009a. Ethanol from Biomass. In: Biswas, S., Basak, P.R., Kaushik, N. (Eds.) Bioprocess and Bioproducts—Emerging Trends. TIFAC, New Delhi, pp. 13–24.
- Sukumaran, R.K., Pandey, A., 2009b. India Country report. In: Eisentraut, A. (Ed.), Global Potential for Sustainable Production of 2nd Generation Biofuels, IEA 2009, p. 26.
- Sukumaran, R.K., Singhania, R.R., Mathew, G.M., Pandey, A., 2009. Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. *Renew. Energy* 34 (12), 421–424.
- Wang, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour. Technol.* 83, 1–11.
- Taherzadeh, M., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int. J. Mol. Sci.* 9, 1621–1651.
- USDE, 2009. Theoretical Ethanol Yield Calculator from Web Site of the Biomass Energy Program. US Department of Energy. www1.eere.energy.gov/biomass/ethanol_yield_calculator.html (accessed 8.04.09).
- van Zessen, E., Weismann, M., Bakker, R.R., Elbersen, H.W., Reith, J.H., den Uil, H., 2003. Lignocellulosic Ethanol, A Second Opinion, Report 2GAVE-03.11. Netherlands Agency for Energy and Environment May 2003. <http://www.novem.nl/default.asp?menuid=10&documentid=34649>.
- Venkatesh, B., Leonardo da Costa, S., Shishir, P.S.C., Derek, M., Lekh, N.S., Chambliss, C.K., Bruce, E.D., 2009. Enzymatic digestibility and pretreatment degradation products of AFEX-treated hardwoods *Populus nigra*. *Biotechnol. Prog.* 25, 365–375.
- Wheals, A.E., Bassoc, L.C., Alves, D.M.G., Amorim, H.V., 1999. Fuel ethanol after 25 years. *Trends Biotechnol.* 17 (12), 482–487.
- White, T., Hindle, C., 2000. Genetic constructs and genetically modified microbes for enhanced production of beta-glucosidase. US Pat. 6015703 (to Iogen Corporation, Ottawa, CA) 18 January, 2000.
- Wisselink, H.W., Toirkens, M.J., Wu, Q., Pronk, J.T., van Maris, A.J.A., 2009. Novel evolutionary engineering approach for accelerated utilization of glucose, xylose, and arabinose mixtures by engineered *Saccharomyces cerevisiae* strains. *Appl. Environ. Microbiol.* 75, 907–914.
- Wyman, C.E., 1996. In: Wyman, C.E. (Ed.), Handbook on Bioethanol: Production and Utilization. Taylor and Francis, Washington DC.
- Wyman, C.E., 2007. What is (and is not) vital to advancing cellulosic ethanol. *Trends Biotechnol.* 25, 153–157.
- Xiao, Z., Zhang, X., Gregg, D.J., Saddler, J.N., 2004. Effects of sugar inhibition on cellulases and beta-glucosidase during enzymatic hydrolysis of softwood substrates. *Appl. Biochem. Biotechnol.* 113–116, 1115–1126.
- Zhang, Y., Zhu, Y., Zhu, Y., Li, Y., 2009. The importance of engineering physiological functionality into microbe. *Trends Biotechnol.* doi:10.1016/j.tibtech.2009.08.006.
- Zhang, Y.H., 2008. Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. *J. Ind. Microbiol. Biotechnol.* 35, 367–375.