Viable feedstock options and technological challenges for ethanol production in India

Anil Kumar Singh, Neelima Garg and Ajay Kumar Tyagi*

Shriram Institute for Industrial Research, 19, University Road, Delhi 110 007, India

Though improvements in processing and technology are important, the fluctuating price of inputs such as molasses, corn, sugar beet, sugarcane, sweet sorghum, starch, etc. and their seasonal availability play an important role in ethanol industry. As a matter of fact, the ethanol industry based on conventional resources has reached its saturation point. Technologies for ethanol production from lignocellulosics are being developed by scientists world over with the objective of exploiting the potential of a resource, which is otherwise considered a waste, to generate energy. The focus has been to produce ethanol in a cost-effective manner, besides aiming to find use of its by-products as food supplements for cattles, etc. Recent developments like adoption of technologies such as dry grind fractionation, which is now commercially viable, would reduce the cost of milling; wet milling being cost-intensive and dry milling requiring smaller plants.

Keywords: Ethanol, feedstock, lignocellulosics, molasses, sugarcane.

URBANIZATION and rapid population growth are responsible for increasing consumption of energy in the world. The worldwide consumption of energy has increased 17-fold in the last century[1]. On the other hand, conventional energy sources (non-renewable) such as fossil fuels are limited and thus cannot meet the demand in the long term. Moreover, uses of fossil fuels also have a negative environmental impact, e.g. increased greenhouse gas emissions. Thus, the major challenge for sustainability world over is to find renewable, efficient, cost-effective and environment-friendly sources of energy that can meet the demand of the transport, energy and industrial sector. Bioethanol, manufactured by fermenting any biomass rich in carbohydrate content (starch, sugar and cellulose), is considered to be one of the alternatives to fossil fuels. The transport sector itself is considered as one of the largest consumer of fuels contributing significantly to environmental pollution[2]. According to the International Energy Agency statistics, the transport sector accounts for about 60% of total global oil consumption. About 20% of CO_2 emission on a global scale is due to transport fuels[2–4].

*For correspondence. (e-mail: aktyagi@shriraminstitute.org)

Bioethanol is emerging as a fuel for cars, which can be used in its anhydrous form. Bioethanol from different renewable sources has already been introduced on a large scale in Brazil, Europe, USA, China and India[5–11]. In 2006, out of the total world production of 48,652 million liters, USA and Brazil together contributed around 70%. The production of ethanol needs a variety of feedstock that can meet the huge demand. At present, major ethanol-producing countries in the world are Brazil, USA, China and India, which produce ethanol from sugarcane, corn, grains and molasses respectively. It is expected that around 33% of the energy needs of Europe and USA for different transportation purposes will be satisfied by converting biomass to biofuels by 2030 (refs 10–13).

Bioethanol production

Feedstock options

The material biomass comes from plants, which utilize solar energy for converting CO_2 and H_2O into sugar. Some plants store the energy in the form of simple sugars, while others store it as complex sugars (starches)[14]. Both of these sugars can be fermented into bioethanol. Another type of resource, i.e. cellulosic biomass is composed of complex sugar polymers, which cannot be directly fermented into bioethanol and need pretreatment and hydrolysis to fermentable sugars. Various categories of feedstock used worldwide for ethanol production are mainly the source of sugars that can be directly fermented. For example, molasses, cane sugar, sweet sorghum, sugar beet and other fruits consisting of sugars and different grains (wheat, corn, rice, sorghum, barley, etc.) and other tubers (sweet potato, cassava, etc.) consisting of starches. Both these types of feedstock are derived from agricultural crops and hence their production would always be a topic of debate on 'food versus fuel'. The other renewable feedstocks with potential to be the source of fermentable sugars are cellulosics and lignocellulosics derived from plant waste materials such as straw, wood, bagasse, etc. Such feedstocks have yet to be exploited for the production of ethanol[15–17].

The feedstocks used for bioethanol production vary throughout the world (Table 1)[18]. In Brazil, sugarcane is preferred; in USA corn is the major crop; across Europe it

is predominantly wheat and barley, and in India, it is sugarcane molasses – a by-product of sugar manufacturing industry[3].

Sugarcane juice: In India, majority of ethanol is obtained from sugarcane molasses. In the future it may also be produced directly from sugarcane juice[19]. Shetimal Sahakari Sanstha Ltd, Kolhapur, Maharashtra is the first plant of 30 kilo litre per day (KLPD) fuel ethanol production directly from sugarcane juice[20–22].

Sweet sorghum: A high-yielding variety, namely Madhura, mainly for ethanol production has been developed[23]. One manufacturing unit of 40 KLPD capacity for ethanol was set-up in 2006, based on sweet sorghum in Medak district, Andhra Pradesh. Depending on the success of this unit, another one with 200 KLPD capacity of biofuels from sweet sorghum is expected soon. It is apparent that in future, sweet sorghum may become the major feedstock for ethanol in India[24].

Tropical sugar beet: This is another high-yielding variety of sugar, which is being tested in thousands of farms in Maharashtra[25]. If successful, the faster growth rate of tropical beet compared to sugarcane may motivate farmers to switch over to the former.

Fruits: These are being used only for production of wine. It will not be viable to use fruits for production of transport fuel.

Grains: These are used only for potable ethanol. It will be a non-viable feedstock of ethanol for transport sector.

Damaged grains: Among these, sorghum is the most frequently used grain for ethanol production, but again for potable liquor.

Tuber: Cassava has been used in South India for potable ethanol production, but this cannot become the major feedstock.

Cellulosics and lignocellulosics: Technology for ethanol production from cellulosics is still under development stage[26]. Among the various feedstocks available, by-products of sugarcane processing industry, i.e. molasses from sugar industry are mainly used for ethanol production in India.

As the 'food versus fuel' issue will not allow utilizing food produce as a source of bioethanol production, alternatives like lignocellulosics need to be exploited. Specific feedstocks of cellulosic biomass under consideration include: Different residues from agriculture; dead trees, tree branches, forestry wastes, chips and sawdust from lumber mills; household garbage, solid waste from municipal and other paper products; wastes from different food processing industries (black liquor, by-product from paper and textile industry); energy crops such as fast-growing grasses and trees being developed for the production of ethanol.

Table 1. Country-wise feedstock used for bioethanol production

Type	Feedstock	Country
Carbohydrate	Sugarcane	India, Brazil, Thailand, Philippines
	Sweet sorghum	China
	Sugar beet	France
Starchy	Corn	USA, UK, China
	Wheat	Germany, Spain, France, UK, China
	Cassava	China, Thailand

Technology options

Presently, there are two main processes through which commercial ethanol is being manufactured.

Synthetic process: Conventional method for production of industrial ethanol is acid catalysed hydration of ethylene produced from petroleum resources. The reaction of ethylene with phosphoric acid is

$$CH_2{=}CH_2 + H_2O \xrightarrow[300°C/60\ Atm]{H_3PO_4} C_2H_5OH.$$

The use of acids as a catalyst alters the manufacturing process due to corrosion, safety and other environmental issues. Advanced technology permits ethanol to be manufactured by the use of zeolite or silica aerogel saturated with phosphoric or tungstic acid. The advantages of the present process are that the reaction can be completed in one stage, the used catalyst can be regenerated, and also concerns (associated with sulphuric acid) about environment, corrosion and safety are diminished. The relative low cost of ethylene from petroleum resource makes this route preferable compared to the fermentation route.

Since ethylene is produced from hydrocarbons, which are also used as fuel, making ethanol as an alternative fuel by this process would not be a good option. But if ethanol can be produced by renewable resources in a cost-effective manner, this can be an alternative fuel for the future. This has become feasible and viable after the experiences of bioethanol in countries like Brazil.

Fermentation process: This process of ethanol (bioethanol) production involves conversion of fermentable sugars into ethanol followed by distillation of ethanol. During fermentation, carbon dioxide is evolved as a by-product. Feedstock that can be used in the fermentation process are: Sugar crops like sugarcane, beetroot, switch grass, etc.; By-products from sugar industry like molasses; grains like wheat, corn, etc.; lignocellulosic biomass.

While some feedstocks are directly fermentable, the others need to be processed to be broken down to fermentable sugar.

Ethanol production from directly fermentable feed stocks: Molasses, produced from the sugar industry, consisting of 45–50% sugars (total reducing sugars; TRS) and is the major feedstock for manufacturing of ethanol in India. On the basis of the efficiency of sugar recovery from sugarcane in sugar mills, molasses of different grades is produced as[12,27–31]: 'A' grade molasses that contains 50% or above TRS; 'B' grade molasses which contains 45–50% TRS; 'C' grade molasses that contains 40–45% TRS.

Due to low cost, high efficiency and ease of fermentation, molasses is widely used in India for the production of ethanol. The most common grade of molasses used in India for ethanol production is 'B' grade[27,28].

Fermentation mechanism: The fermentable carbohydrates in molasses are sugars, principally sucrose, which are converted to mixture of glucose and sucrose (invert sugar) for fermentation into ethanol by yeast. The process of ethanol production is given below.

Maintenance of yeast cultures and their development to plant stage: Distillery yeasts are usually strains of *Saccharomyces cerevisiae* and are commonly maintained in the laboratory on malt or molasses agar slants and transferred at monthly intervals. Yeast cultures are successfully propagated in the laboratory in two stages: (i) in 12–15% sterilized malt extract medium and (ii) in sterilized mash, the sugar being supplied by malt extract and molasses. From this second stage, yeast cells are aseptically transferred to the first plant stage.

The first plant stage is also known as the preseed stage. The mash for this stage and the following seed stage consists entirely of sterilized dilute molasses and inorganic nutrients. The sugar concentration in these stages is usually 8–12%. The final seed stage is used to inoculate the final fermentors. Usually an inoculum of 2–4% by volume of active seed yeast is used for inoculation of final fermentors[16,17,29,31].

Plant fermentation: For the plant mash, molasses is diluted with water to give a sugar concentration of 14–18%. Also, pH of the mash is adjusted to 4–5, if required, by addition of sulphuric acid. Although the optimum pH for maximum efficiency varies with the molasses used, an initial pH of 4.8–5.0 is usually considered the best. Hydrochloric or lactic acid may be used in place of sulphuric acid. Some amount of ammonium salt (0.227–1.359 kg per 3785 litre) is added to the mash to increase the rate and efficiency of fermentation.

The plant mash is then pumped directly into the fermentor. The fermentor is seeded when it is one-eighth to one-fourth full, with a large volume of active yeast (2–4% of the final volume) to allow development during the entire filling period, which may amount to 8 h.

The chemical process involves: (i) Conversion of sucrose to glucose and fructose

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Invertase}} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}.$$

(ii) Fermentation of sugars to ethanol with the generation of carbon dioxide and heat

$$\underset{\text{Sugar}}{C_6H_{12}O_6} \xrightarrow{\text{Zymase}} \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2} + \underset{\text{Heat}}{23.5\ \text{kcal}}.$$

Types of fermentation: Four processes, i.e. batch, continuous, fed-batch and semi-continuous are used to ferment molasses.

In batch fermentation, yeast culture and substrate are charged together with nutrients in the bioreactor. The batch process is commonly used in most of the ethanol produced today in India. The operation cost is low, since it can be accomplished by less skilled labour and low capital investment[4,29,32].

In the continuous process, culture media, feed and nutrients are discharged continuously into the agitated fermentation chamber and the product is collected continuously from the top of the bioreactor. The product contains ethanol, yeast cells and residual sugar[13,30,33].

The fed-batch operation technique is a combination of both batch and continuous techniques and has become popular. Here, the substrate, enzymes, nutrients and yeast culture are fed at regular intervals, while the effluent is removed at a certain interval. The main advantage of this technique is the prevention, inhibition and catabolic repression mainly due to intermittent feeding of the substrate.

In the semi-continuous process, a portion of the culture is taken out at regular intervals and fresh media added into the system. It is essential to maintain constant volume during the continuous process, whereas volume variation occurs in semi-continuous and fed-batch processes. Semi-continuous process has some advantages over batch and continuous process, which are as follows: Less time-consuming since cleaning and sterilization are not required; Separate inoculation chamber is not required; Lesser controls are required.

However, this method also has some disadvantages, such as risk of mutation and contamination and slightly higher investment costs for establishment of larger reactor[32].

Fermentation conditions and processes: Since the reaction is exothermic, to maintain optimum temperature of the reaction chamber at 25–32°C, the fermenter is cooled by spraying water, using cooling coil, or by external coolers. The amount of heat liberated during fermentation

is in agreement with the theoretical values. The heat produced from a fermentation involving 67,950 kg of sugar is 39,000,000 Btu (ref. 34).

Fermentation begins after the fermentor is filled and is active after 2–4 h; it typically takes 48–80 h for completion. After fermentation is complete, the resulting broth contains ethanol (6–8%). Yeast culture obtained from cell free broth is taken for distillation once fermentation is completed.

For ethanol distillation system by Indian distilleries, generally 6–9 fermenters are used for ensuring continuous feed[35]. Over 100 distilleries in India are producing ethanol by continuous process, due to its higher efficiency, i.e. 89–90% (80–84% for batch process), less water consumption and ease of operation[3,13,36].

Distillation: Preheated (90°C) cell-free fermented broth is sent to the de-gasifying chamber of the analyser section. Any trapped gases are removed by bubble-cap fractionating column from the liquor. To the rectifying column, ethanol vapours from the analyser chamber are further taken, 94–96% rectified ethanol is trapped, cooled and collected by reflux action.

Besides molasses, other feedstocks such as sugar beet, sugarcane and sweet sorghum can also be used as sources of sugar for ethanol production[30,31,37–42].

Dehydration: As a fuel, water must be removed completely from the bioethanol. From distillation, the purity is limited to 95–96%, due to the formation of low-boiling water–ethanol azeotrope. The 96% ethanol v/v (95% w/w) and 4% v/v water (5% w/w) mixture may be used as a fuel only in FLEXI-FUEL vehicles and it is called hydrated ethanol[30,42–44].

For ethanol to be used as fuel in conventional vehicles, it is necessary to remove water from it, i.e. to make ethanol anhydrous to a minimum of 99.7% w/w purity level. Currently, the most widely used technique for the production of anhydrous ethanol is the physical adsorption (of water) process using molecular sieves. A molecular sieve contains a series of small beads that adsorb all the remaining water from hydrated ethanol. Ethanol molecules are too large to enter the sieves; thus the dehydration step produces pure ethanol[14,16,29,31].

Another method by which dehydration of ethanol is achieved uses hydrocarbon benzene (entrainer) in azeotropic distillation. Addition of benzene breaks the water–ethanol azeotrope and ethanol off with benzene leaving water. A third method involves the use of calcium oxide as desiccant. Prior to shipping ethanol to the gasoline distribution centre for blending, a small amount of gasoline (~5%) is added to denature the ethanol, making it unfit for drinking[3,12,30,42,45].

Ethanol production from indirectly fermentable feedstocks: All grains like wheat, rice, corn and barley are good sources of starch. The conversion of corn and other food/feed crops into ethanol by fermentation is a well-known and established technology which involves three steps: Pretreatment – milling, extraction and hydrolysis to form a solution of fermentable sugars; Fermentation of sugars to ethanol; Separation and purification of ethanol to anhydrous ethanol.

Production of ethanol from grain sources includes grain milling, starch hydrolysis and inoculation of yeast cell. The remaining spillage from ethanol production is processed to produce distiller's dried grains and solubles (DDGS), a good ingredient for animal feed.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6,$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy (stored as ATP)}.$$

In USA, corn is the principal feedstock for ethanol production. The ethanol yield from a large production plant is about 1 litre from 2.69 kg of corn grain[46].

On the basis of methodology of milling, the process can be divided into two types, i.e. dry and wet milling.

Dry milling: This includes the following processes:

Grinding: Whole grain is utilized in this method. It is ground into flour and referred to as meal, which is processed out without separation into the various component parts of the grain[47].

Cooking: Meal is converted into slurries with water to form mash and enzymes are mixed for converting starch to dextrose. For pH control, NH_3 is added which also acts as a nutrient to the yeast. The mash is finally processed under certain temperature and pressure called cooking, which makes mash suitable for fermentation.

Fermentation: After cooling, the mash is transferred to the fermentor where yeast culture is added and the conversion of sugar to ethanol and CO_2 takes place. The fermentation process generally takes about 40–50 h.

Distillation: The resulting beer is transferred to the distillation chamber after fermentation, where ethanol is concentrated to 190 proof by removing water and impurities of aldehyde, ketone and other higher alcohols. To obtain 200 proof ethanol, the purified ethanol is passed through a molecular sieve system.

Wet milling: This includes the following processes.

Steeping: The grains are soaked in wet milling for 24–48 h in a mixture of dilute sulphurous acid (H_2SO_3) and water. This steeping facilitates breakdown of the grains into separable components[47–49].

Table 2. Technological challenges involved in bioethanol production

Production process	Process type/step	Challenge
Synthetic alcohol	Hydration of ethylene	Ethanol from synthetic route cannot be the alternative to fossil fuels.
Directly fermentable feedstock (molasses, sugarcane, sugar beet and sweet sorghum)	Batch	Need frequent cleaning and sterilization. Poor efficiency (80–84%) as against 89–90% in continuous. Needs a separate inoculum vessel. More controls are required
	Continuous	High risk of contamination and mutation due to long cultivation period.
	Semi-continuous	Higher investment costs for larger reactor.
Indirectly fermentable feedstock (starchy and industrial waste)	Milling and hydrolysis	Need costly pretreatment, steeping, milling and hydrolysis to convert starchy material into fermentable substrates.
Ethanol from lignocellulosic biomass	Pretreatment	Extensive energy, chemical/enzyme demand. Reduction in cost of pretreatment reactor. To curtail pollutants such as black liquor of lignin.
	Dilute acid hydrolysis	Toxic degradation products like furan from sugars, aliphatic acids from hemicellulose acetyl groups, and phenolics from lignin that interfere with fermentation.
Ethanol from lignocellulosic biomass	Concentrated acid hydrolysis	Cost-effective acid recovery.
	Microbial fermentation of hexoses and pentoses to ethanol	Microorganisms capable of fermenting both hexoses and pentoses with effective rate of conversion.
	Direct conversion of cellulose into ethanol	To minimize by-products like acetate and lactate that lower the efficiency of the process.
Ethanol from gasification of lignocellulosic biomass	Gasification	Extensive energy demand. Combustion needs sophisticated controls to obtain the desired composition of syngas.
	Fermentation	Needs special kind of fermenter that can support bacteria, *Clostridium ljungdahlii*.
	Catalytic conversion	To arrest the formation of by-products like methanol, butanol and propanol.

Grinding and separation: Steeped corn is ground in wet condition through a series of grinders and four products are separated: (i) corn germ (embryo portion of seed), (ii) fibre (outer tough covering of corn seed), (iii) gluten (protein part of corn seed) and (iv) starch (carbohydrate part of corn seed).

Product (i) is used for corn oil production. Products (ii) and (iii) are segregated using centrifugal, screen and hydrocyclonic separators, both constitute a good feed for livestock industry. Heavy steep water left after extraction of the above four components is generally used in feed preparation and melting ice on roads; hence, it is also known as ice bran[50,51].

The last product, i.e. (iv) starch, can be processed in one of the three ways: processed into corn syrup, dried and sold as corn starch or fermented into ethanol[12,13,29,31,42,50]. The processes of cooking, fermenting and distillation are same as mentioned earlier in the process of dry milling.

Comparative studies between wet and dry milling showed that ~5% more ethanol yields with wet milling process, dry milling process and the average oil content of germ was 10% more[17,18,42].

Pretreatment cost of directly fermentable sugar containing materials is less, whereas indirectly fermentable starchy materials need costly pretreatment to convert them into fermentable substrates[52]. Besides this, such resources cannot be considered as sustainable because they would be used as food first and not for fuel.

However, it may be noted that the lignocellulosics can also be converted into fermentable form similar to the manner in which starchy products can be processed. Moreover, this would involve certain technological challenges related to extracting and processing of the fermentable sugar that can then be converted into bioethanol[53–65].

Technological challenges

Based on the various technological options discussed in this article for the production of ethanol, Table 2 provides

a summary of the challenges that need to be addressed by technologists[66,67].

Challenges involved in large-scale bio-ethanol production

Socio-economic challenges: These include the following:

Food prices: The role of biofuels in increasing the price of food has already been debated earlier. It is estimated that ~30% increase in food prices can be attributed to the increased production of bioethanol in place of food, as more grains are required for more ethanol production causing increased price of grains. The prices of raw materials are expected to grow further by 30–40% by 2020 (refs 68–82).

Tenure and conflicts: The production of more ethanol requires more land and water. Such type of agricultural shift can reduce the production of other grains causing scarcity of food. The marginal lands that are being targetted are often inhabited and used by rural farmers for various subsistence purposes[83–92].

Lower fuel economy: The energy content of ethanol is less than gasoline; so increasing the percentage of ethanol in gasoline, most likely results in lower fuel economy. However, using 5–10% ethanol in the blend will have little or no impact on fuel economy[93–104]. Further increasing the ethanol by ~20% reduces fuel economy ~1–3% (refs 105–114).

Environmental challenges: These includes the following:

Soil and water: It has been observed that mostly crops related to biofuel are highly water-intensive. Crops like tropical sugar beet and sweet sorghum can grow on soil containing less water content, but may require fertilizers and irrigation to become commercially profitable crops. In Brazil, sugarcane cultivation is a big consumer of agricultural pesticides, causing pollution of water ecosystems[115].

Deforestation, climate change and ecosystem services: Deforestation often takes place due to large-scale monoculture biofuel production, which is a major threat to biodiversity. Intact savannas, forest and wetlands provide ecosystem benefits to people such as protection against extreme weather events, carbon storage, erosion and pollination needed for the production of other crops[115].

Conclusion

Considering the multi-feed approach of bioethanol production as adopted worldwide depending upon the availability of a particular feedstock in a country or even a region, no universal process can be adopted. The seasonal nature of the renewable feedstock also forces one to adopt the multi-feed approach for ethanol production throughout the year. Irrespective of the technological and feedstock options available, a cost-effective and environment-friendly process is needed for the industry as well as society. Above all, an immediate priority of research in the field of ethanol production is to develop processes for the conversion of lignocellulosics into bioethanol with tenfold reduction in the cost by identifying microorganisms that can ferment both five- and six-carbon sugars and enzymes that break down cellulose directly into ethanol.

It is apparent that for the conversion of biomass to ethanol, the first step may be radiation processing of the biomass. So as far as the other steps of manufacturing ethanol from the residue biomass are concerned, the process seems well established. The emphasis would be on the improvement in the efficiency of the fermentation technology. The research efforts would therefore be directed towards realizing the expertise acquired so far in all the above-mentioned challenging areas.

1. Demirbas, A., *Prog. Energy Combust.*, 2007, **33**, 1–18.
2. International Energy Agency (IEA), World energy outlook, Paris, OECD/IEA, 2008, pp. 1–578.
3. Guo, Y., Yeh, T., Song, W., Xu, D. and Wang, S., *Renew. Sustain Energy Rev.*, 2015, **48**, 776–790.
4. Behera, S., Mohanty, R. C. and Ray, R. C., *Appl. Energy*, 2010, **87**, 2352–2355.
5. Balat, M. and Balat, H., *Appl. Energy*, 2009, **86**, 2273–2282.
6. Balat, M., Balat, H. and Oz, C., *Prog. Energy Combust.*, 2008, **34**, 551–573.
7. Sanchez, O. J. and Cardona, C. A., *Bioresour. Technol.*, 2008, **99**, 5270–5295.
8. Cardona, C. A. and Sanchez, O. J., *Bioresour. Technol.*, 2007, **98**, 2415–2457.
9. Neves, M. A., Kimura, T., Shimizu, N. and Nakajima, M., *Global Sci. Books*, 2007, **1**, 1–14.
10. Baras, J., Gaćeśa, S. and Pejin, D., *Chem. Ind.*, 2002, **56**, 89–105.
11. Report on alternative fuels for road transportation and on a set of measures to promote the use of biofuels, Commission of the European Communities, Brussels, Belgium, 2001, pp. 1–50.
12. Gonçalves, F. A., Santos, E. S. and Macedo, G. R., *Renew. Sustain Energy Rev.*, 2015, **50**, 1287–1303.
13. Puri, M., Abraham, R. E. and Barrow, C. J., *Renew. Sustain Energy Rev.*, 2012, **16**, 6022–6603.
14. http://www.energy.gov
15. http://www.biofuelstp.eu
16. Yoon, M. H., Lee, Y. W., Lee, C. H. and Seo, Y. B., *Bioresour. Technol.*, 2012, **126**, 198–201.
17. Degliuomini, L. N., Biset, S., Luppi, P. and Basualdo, M. S., *Int. J. Hydrogen Energy*, 2012, **37**, 3108–3129.
18. Rijal, B., Biersbach, G., Gibbons, W. R. and Pryor, S. W., *Appl. Biochem. Biotechnol.*, 2014, **174**, 845–854.
19. Alexdender, K. M., Ethanol production from sugarcane in India: viability, constraints and implications, M Sc thesis, Department of Natural Resources and Environmental Sciences, University of Illinois, USA, 2010.
20. http://www.novozymes.com

21. http://www.cfr.org/
22. http://www.ethanolindia.net/
23. http://iopscience.iop.org/1748-9326/4/4/044005/fulltext/
24. http://www.tnau.ac.in/
25. Reddy, M. V., Raju, T. N., Sharma, S. B., Nene, Y. L., McDonald, D., Pande, S. and Sharma, M., *Handbook of Pigeonpea Diseases (Revised). Information Bulletin No. 42,* Technical Report. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 2012.
26. http://www.oregon.gov/pages/index.aspx
27. http://www.academia.edu/5991944/Molasses_Types_and_its_Utilization1
28. George Philander, S. (ed.), *Encyclopedia of Global Warming and Climate Change*, SAGE Publication Inc, California, USA, 2008.
29. Li, P. and Zhu, M., *Bioresour. Technol.*, 2011, **102**, 10471–10479.
30. Zhou, S. and Runge, T. M., *Bioresour. Technol.*, 2015, **193**, 288–296.
31. Saini, R., Saini, J. K., Adsul, M., Patel, A. K., Mathur, A., Tuli, D. and Singhania, R. R., *Bioresour. Technol.*, 2015, **188**, 240–246.
32. Caylak, B. and Vardar Sukan, F., *Turk J. Chem.*, 1998, **22**, 351–359.
33. Maiorella, B., Wilke, Ch R. and Blanch, H. W., *Adv. Biochem. Eng.*, 1981, **20**, 9–31.
34. Underkofler, L. A. and Hickby, R. J. (eds), *Industrial Fermentations, Vol 1*, Chemical Publication Co, Cleveland, USA, 1954.
35. The Energy and Resources Institute, *Teri Energy Data Directory and Year Book*, 2003–04.
36. http://www.ethanolindia.net/sugarind.html
37. Phowchinda, O., Delia-Dupuy, M. L. and Strehaiano, P., Seminar on biotechnology: an essential tool for future development? In Proceedings of the Ninth Annual Meeting of the Jhai Society for Biotechnology and the Second JSPS-NRCT-DOST-LIPP-VCC, 1997.
38. Rudolph, K., Owsianowski, R. and Tentscher, W., Director production of ethanol from sugarcane. *Int. Sugar J.*, 1979, **81**, 253.
39. Hill, F. J., Levellen, R. T. and Skoyen, I. O., *Calif. Argic.*, 1990, **44**, 14–16.
40. Gibbons, W. R., Westby, C. A. and Dobbs, T. L., *Appl. Environ. Microbiol.*, 1986, **51**, 115–122.
41. Kudoh, Y., Nagasawa, M. and Sagisaka, M., Inventory analysis of bio-ethanol production from energy crops. National Institute of Advance Science and Technology, Japan, 2008.
42. Arora, R., Behera, S. and Kumar, S., *Renew. Sustain Energy Rev.*, 2015, **51**, 699–717.
43. http://www.heblends.com/index.php?option=com_content&task=view&id=10&Itemid=25
44. http://www.eac-quality.net/
45. US 8172987 B2, Low-energy extractive distillation process for dehydration of aqueous ethanol, 2012.
46. Pimentel, L. S., *Biotechnol. Bioeng.*, 1980, **22**, 1989–2012.
47. http://www.ethanolrfa.org/
48. Khoo, H. H., *Renew. Sustain Energy Rev.*, 2015, **46**, 100–119.
49. Liu, Z. *et al.*, *Bioresour. Technol.*, 2013, **135**, 292–303.
50. USEPA, Composition and behavior of fuel ethanol, United States Environmental Protection Agency, 2009.
51. Devis, K. S. and Milling, C., In Minnesota Corn Growers Association Technical Symposium, Minnesota, USA, 11 September 2001.
52. Sun, Y. and Cheng, J., *Bioresour. Technol.*, 2002, **83**, 1–11.
53. Wyman, C. E., *Bioresour. Technol.*, 1994, **50**, 3–16.
54. Kuhad, R. C., Kuhar, S., Kapoor, M., Sharma, K. K. and Singh, A., *Lignocellulose Biotechnology, Future Prospects*, I.K. International Publishing House Pvt Ltd, New Delhi, 2007.
55. Knauf, M. and Moniruzzaman, M., *Int. Sugar J.*, 2004, **106**, 147–150.
56. McMillan, J. D., Pretreatment of lignocellulosic biomass. In *Conversion of Hemicellulose Hydrolyzates to Ethanol* (eds Himmel, M. E., Baker, J. O. and Overend, R. P.), American Chemical Society Symposium, Washington, DC, 1994, pp. 292–324.
57. Shafizadeh, F. and Bradbury, A. G. W., *J. Appl. Polym. Sci.*, 1979, **23**, 1431–1442.
58. Mes-Hartree, M., Dale, B. E. and Craig, W. K., *Appl. Microbiol. Biotechnol.*, 1988, **29**, 462–468.
59. Holtzapple, M. T., Lundeen, J. E. and Sturgis, R., *Appl. Biochem. Biotechnol.*, 1992, **34/35**, 5–21.
60. Tengerdy, R. P. and Nagy, J. G., *Biol. Wastes*, 1988, **25**, 149–153.
61. Dale, B. E., Henk, L. L. and Shiang, M., *Dev. Ind. Microbiol.*, 1984, **26**, 223–233.
62. Roy, S., Ghosh, S. and Das, D., *Int. J. Hydrog. Energy*, 2012, **37**, 15867–15874.
63. Zheng, Y. Z., Lin, H. M. and Tsao, G. T., *Biotechnol. Prog.*, 1998, **14**, 890–896.
64. Mais, U., Esteghlalian, A. R., Saddler, J. N. and Mansfield, S. D., *Appl. Biochem. Biotechnol.*, 2002, **98**, 815–832.
65. Tassinari, T. and Macy, C., *Biotechnol. Bioeng.*, 1977, **19**, 1321–1330.
66. Mussatto, S. I. *et al.*, *Biotechnol. Adv.*, 2010, **28**, 817–830.
67. Amorim, H. V., Lopes, M. L., Oliveira, J. V., Buckeridge, M. S. and Goldman, G. H., *Appl. Microbiol. Biotechnol.*, 2011, **91**, 1267–1275.
68. Sidiras, D. K. and Koukios, E. G., *Biomass*, 1989, **19**, 289–306.
69. Kumukura, M., Kojima, T. and Kaetsu, I., *Biomass*, 1982, **2**, 299–308.
70. Lafitte-Trouque, S. and Forster, C. F., *Bioresour. Technol.*, 2002, **84**, 113–118.
71. Kennedy, K. J., Thibault, G. and Droste, R. L., *Water SA*, 2007, **33**, 261–270.
72. Park, B., Ahn, J. H., Kim, J. and Hwang, S., *Water Sci. Technol.*, 2004, **50**, 17–23.
73. Eskicioglu, C., Terzian, N., Kennedy, K. J., Droste, R. L. and Hamoda, M., *Water Res.*, 2007, **41**, 2457–2466.
74. Keller, F. A., Hamilton, J. E. and Nguyen, Q. A., *Appl. Biochem. Biotechnol.*, 2003, **27–41**, 105–108.
75. Nelly, W. C., *Biotechnol. Bioeng.*, 1984, **20**, 59–65.
76. Ben-Ghedalia, D. and Shefel-G., *J. Agric. Sci.*, 1983, **100**, 393–400.
77. Vidal, B. F. and Molinier, J., *Biomass.*, 1988, **16**, 1–17.
78. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R. and Lee, Y. Y., *Bioresour. Technol.*, 2005, **96**, 1959–1966.
79. Sivers, M. V. and Zacchi, G. A., *Bioresour. Technol.*, 1995, **51**, 43–52.
80. Esteghlalian, A., Hashimoto, A. G., Fenske, J. J. and Penner, M. H., *Bioresour. Technol.*, 1997, **59**, 129–136.
81. Clark, T. and Mackie, K. L., *J. Chem. Biotechnol.*, 1984, **34**, 101–110.
82. Converse, A. O., Kwarteng, I. K., Grethlein, H. E. and Ooshima, H., *Appl. Biochem. Biotechnol.*, 1989, **20/21**, 63–78.
83. Vaccarino, C., Lo Curto, R. B., Tripodo, M. M., Bellocco, E., Laganfi, G. and Patan, R., *Biol. Wastes*, 1987, **20**, 79–88.
84. Fan, L. T., Gharpuray, M. M. and Lee, Y. H., *Cellulose Hydrolysis Biotechnology Monographs*, Berlin, Springer, 1987, p. 57.
85. Kim, T. H. and Lee, Y. Y., *Bioresour. Technol.*, 2006, **97**, 224–232.
86. Pasquini, D., Pimenta, M. T. B., Ferreira, L. H. and Curvelo, A. A. D. S., *J. Supercrit. Fluid*, 2005, **36**, 31–39.
87. Fan, L., Lee, Y. and Gharpuray, M., *Adv. Biochem. Eng. Biotechnol.*, 1982, **23**, 158–183.
88. Gregg, D. J. and Saddler, J. N., *Biotechnol. Bioeng.*, 1996, **51**, 375–383.
89. Azzam, A. M., *J. Environ. Sci. Health*, 1989, **24**, 421–433.

90. Bon, Elba, P. S. and Ferrara, Antonieta, M., Bioethanol production via enzymatic hydrolysis of cellulosic biomass. In Seminar, Rome, 2007.
91. Boominathan, K. and Reddy, C. A., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 5586–5590.
92. Lloyd, T. A. and Wyman, C. E., *Bioresour. Technol.*, 2005, **96**, 1967–1977.
93. Beall, D. S., Ohta, K. and Ingram, L. O., *Biotechnol. Bioeng.*, 1991, **38**, 296–303.
94. Ranatunga, T. D., Jervis, J., Helm, R. F., McMillan, J. D. and Hatzis, C., *Appl. Biochem. Biotechnol.*, 1997, **67**, 185–195.
95. Taherjadeh, M. J., Gustafsson, L., Niklasson, C. and Liden, G., *J. Biosci. Bioeng.*, 1999, **87**, 169–174.
96. Martinez, A., Rodriguez, M. E., York, S. W., Preston, J. F. and Ingram, L. O., *Biotechnol. Bioeng.*, 2000, **69**, 526–536.
97. Rodrigues, R. C. L. B., Felipe, M. G. A., Almeida-e-silva, J. B., Vitola, M. and Gomez, P. V., *Braz. J. Chem. Eng.*, 2001, **18**, 299–311.
98. Nilvebrant, N., Reimann, A., Larsson, S. and Jonsson, L. J., *Appl. Biochem. Biotechnol.*, 2001, **91–93**, 35–49.
99. Martin, C., Galbe, M. and Wahlbon, C. F., *Enzyme Microb. Technol.*, 2002, **31**, 274–282.
100. Lopez, M. J., Nichols, N. N., Dien, B. S., Morano, J. and Bothast, R. J., *Appl. Microbiol. Biotechnol.*, 2004, **64**, 125–131.
101. US 5188673, Concentrated sulfuric acid process for converting lignocellulosic materials to sugars, 1993.
102. Roehr, M., *The Biotechnology of Ethanol – Classical and Future Application*, Wiley-VCH, Weinheim, 2001, p. 157.
103. http://www.iisc.ernet.in/currsci/jul10/articles14.htm
104. Krishnan, M. S., Ho, N. W. and Tsao, G. T., *Appl. Biochem. Biotechnol.*, 1999, **77–79**, 373–388.
105. Jin, Y. S., Laplaza, J. M. and Jeffries, T. W., *Appl. Environ. Microbiol.*, 2004, **70**, 6816–6825.
106. Toivari, M. H., Salusjarvi, L., Ruohonen, L. and Penttila, M., Endogenous xylose pathway in Saccharomyces cerevisiae. *Appl. Environ. Microbiol.*, 2004, **70**, 3681–3686.
107. Karhumaa, K., Hahn-Hagerdal, B. and Gorwa-Grauslund, M. F., *Yeast*, 2005, **22**, 359–368.
108. Kuyper, M., Toirkens, M. J., Diderich, J. A., Winkler, A. A., Van Dijken, J. P. and Pronk, J. T., *Biotechnol. Bioeng.*, 2005, **87**, 90–98.
109. Toivola, A., Yarrow, D., Van-den-bosch, E., Van-dijken, J. P. and Sheffers, W. A., *Appl. Microbiol. Biotechnol.*, 1984, **47**, 1221–1223.
110. Dien, B. S., Nichols, N. N., O'Bryan, P. J. and Bothast, R. J., *Appl. Biochem. Biotechnol.*, 2000, **84/86**, 181–196.
111. Roy, S., Gudi, R. D., Venkatesh, K. V. and Shah, S., *Proc. Biochem.*, 2001, **36**, 713–722.
112. Öhgren, K., Bura, R., Lesnicki, G., Saddler, J. and Zacchi, G., *Proc. Biochem.*, 2007, **42**, 834–839.
113. http://www.syntecbiofuel.com/Biofuel_Technologies.html
114. http://blogs.consumerreports.org/cars/2008/03/ethanol-e85.html
115. SwedBio, Swedish EIA Centre, Biofuels-Potential and Challenges for Developing Countries, 2009.

Received 6 June 2015; revised accepted 23 March 2016

doi: 10.18520/cs/v111/i5/815-822