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Review Article

BIOTECHNOLOGY FOR BIO FUELS: LIGNOCELLULOSIC ETHANOL PRODUCTION

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ABSTRACT
Biomass for fuels from renewable sources has been regarded as a feasible solution to the energy and environmental problems in the foreseeable future. Ethanol and biodiesel are predominantly produced from sugarcane, corn kernels, hydrocarbonsor soybean oil. Besides this another bio fuel feedstock, lignocelluloses - the most abundant biological material on earth is also being explored. Wheat straw, corn husks, prairie grass, discarded rice hulls or trees, agri-waste provide a source of lignocellulose material. Recently cellulosic bio fuels provide promising sequester and convert CO_2. The race is on to optimize the technology that can produce biofuels from lignocelluloses sources more efficiently—and biotech companies are in the running. Present review provides state of art report on the lignocellulose as source for biofuels.
Keywords: Bio fuel, Ligno cellulose, Cellulose, Hemi cellulose, Enzymes, Ethanol

INTRODUCTION

Biomass

Biomass is defined as the collection of all organic matter composing biological organisms. The main components utilized for biofuel production are sugars and lipids. The major components of plant secondary cell walls are cellulose, hemi cellulose and lignin[1]. Bio fuels offer one of the best alternative options as they have much lower life cycle GHG emissions compared to fossil fuels. These are liquid fuels derived from renewable biological sources[2-6]. One of the directives of European Union (2009/28/CE) imposes aquota of 10 % for bio fuel sonall traffic fuel until 2020[7]. The most common renewable fuel is ethanol, which is produced from direct fermentation of sugars e.g. sucrose of sugarcane or sugar beet or polysaccharides, starch from corn and wheat grains[8].

Bio fuels

US Department of Energy (DOE) has called for 30 % of today's fuel use to be supplanted by 2030 with ethanol. In that scenario, much of the fuel is slated to come from ligno celluloses. However lingo cellulosic conversion is about three- to fourfold more expensive than a corn grain ethanol plant with the same yield. However active researches are going on in this field[56].

What are available Biomass Resources for Bio fuel Production

Mainly three types of crops are used for bio fuel production:

- Hydrocarbon yielding plants
- Oil yielding plants
- Energy crops for starch and ligno cellulosic material from plants.

Hydrocarbon yielding plants are obtained from wastelands and can be cultivated in saline and alkaline soils unfit for cultivation of other crops[2]. Kumar[9] examined the possibility of using *Calotropis procera* for bio fuel. Non edible oil as well as edible oil also yields biodiesel on trans esterification. The feedstocks for Ethanol production worldwide are sugar cane (in Brazil and other tropical locations) and corn for ethanol and oil palm, soybean and canola or rapeseed for biodiesel. Europeans also use wheat and sugar beets for ethanol production. The alternative use of ligno cellulosic wastes or plant biomass grown on marginal lands or desert areas might represent a promising approach to mitigate the well known competition phenomena for land and food use (see review[10,11]. A large number of species have been suggested as good sources for cellulosic material for biofuel. Several perennial forage grasses in particular are salt-tolerant and easy to manage[12,13]. A perennial, Switch grass (*Panicum virgatum* L.) does not require annual tillage and planting and can be done on reserve lands[14,15] for its value as forage and a bio energy feedstock. Progress in breeding for useful variation in cell wall composition is also possible[16]. Agri residue and municipal waste can be used for methano genesis. Methane and carbon dioxide are the products of second generation fuel. Gasification of coal converts it into sugars which can directly be used as fuel. Cellulosic biofuels have been reviewed recently[17-24]. The present review compiles state of art work on ligno cellulosic biomass conversion technologies.

Strategy for biomass to biofuel

- First generation bio fuel: It included sugar cane, starch seeds, oil seeds and salt and drought resistant hydrocarbon yielding plants for growing in wastelands.
- Second and third generation bio fuels: It included ligno cellulosic biomass agricultural waste and conversation technologies and altering host material and /or developing new enzyme systems[25].
- Metabolic engineering for entire product.
- Industrial application of bio fuel inclusive of related bio products of commercial value from fourth generation products.

Second and third generation bio fuels

It includes Ligno cellulosic biomass, agricultural waste and conversion technologies for this have been worked out. In our previous review the conversion technology was presented[26].

Ligno cellulosic feedstock

Various species have been suggested as good sources for cellulosic material for bio fuel several perennial forage grasses in particular are salt-tolerant and easy to manage[12]. Ligno cellulosic materials are regarded as good candidates for the second-generation ethanol production[27-28] even if cellulose, being embedded with hemicelluloses and lignin in the plant cell wall, has a partial crystalline structure and low accessibility. Thus, adequate, but costly pretreatments are needed to enable its saccharification[29]. Changes in lignin composition have been achieved e.g. transgenic poplar and alfalfas have been produced with reduced lignin accumulation. These plants have reduced lignin content: from 17.6 % to ~14 % in alfalfa and from 20.6 % to 12.8 % in poplar[17].

Ethanol

Ethanol, the most common renewable fuel production by fermentation, has a long history dating back several thousand years. It is produced by direct fermentation of sugars (e.g. from sucrose of sugarcane or sugar beet) or polysaccharides (e.g. starch from corn and wheat grains)[8]

Cellulosic bio fuels

Biological systems utilize photosynthesis to capture and store solarenergy in the form of chemical bonds in biomass[30]. Plants produce about 180 billion tons of cellulose per year globally, making this polysaccharide the largest organic carbon reservoir on earth. Conversion technologies for cellulose into ethanol require special treatment as lignocellulose matter which is highly recalcitrant. A physicochemical pre treatment step is essential to break the robust structure of the ligno cellulosic material in order to increase the accessibility of cellulose and hemi cellulose polymers to cellulolytic enzymes. This facilitates the bio-ethanol conversion. Different methods of pretreatment e.g. physical, thermal, chemical and biological have been reviewed and discussed[26]. Pretreatment with steam explosion process, with a partial de polymerization and dissolution of the hemicelluloses[31] was considered to be best. The current trend is to run the processes at high substrate concentrations; a technology that is known by several different expressions and reviewed by Koppram *et al*[25]. However, the study reveals that no specific pretreatment method can be directly adopted for any ligno cellulosic feedstock without proper pilot plant research due to the considerable number of affecting parameters and amount of variance involved[26].

Microorganisms

Traditionally, ethanol is produced in the yeast *Saccharomyces cerevisiae* or the proteobacteria *Zymomonas mobilis*[32] Santi *et al*[11] reported special strains of *Saccharomyces cerevisiae* Zymaflore F15 for ethanol production. The natural pathways for ethanol production from sugars in *S. cerevisiae* and *Z. mobilis* have led to yields exceeding 95 % of theoretical maximum, which is 0.51 g of ethanol per g of glucose. However further improvement mainly resides in broadening the substrate range, enhancing resistance to product toxicity and increasing robustness in various process conditions. However natural ethanologenic hosts *S. cerevisiae* and *Z. mobilis* lack the ability to ferment pentoses, which are significant hydrolysis products of ligno cellulosic biomass. To tackle this problem, one possibility is to introduce pentose-metabolizing pathways into *S. cerevisiae*[33-35] and *Z. mobilis*[36]. Koppram, *et al*[25] reviewed cost-competitive high-gravity (HG) process of ligno-cellulosic bio fuel production with minimal effects on the environment. The microorganisms have been developed resistant to many stress factors affecting the cells during HG ligno cellulosic bio fuel production using genome shuffling technique to improve the acid tolerance of *S. cerevisiae*[37]. The over expression of genes (e.g., TAL1, TKS1, ERG2, PRS3, and RAV1) that confer resistance to inhibitors has also gained interest[31,38,39]. However, one can express the ethanologenic pathways into *E. coli*, whose broad range of carbohydrate metabolizing capacity makes it a top candidate for biocatalyst engineering[40].

Technological advancement

Bioconversion

The bioconversion technologies for liquid fuel production have lower capital costs than thermal conversion methods. The key steps in bioconversion of lignocelluloses to fuels are size reduction, pretreatment, hydrolysis and fuel production Biomass transportation costs are reduced by up scaling the processing plants through technological innovations.

Pretreatment and hydrolysis

Ligno cellulose matter is highly recalcitrant and needs suitable pretreatment to degrade lignin and ease the way for cellulose and hemi celluloses digestion. The latest pretreatment research focuses on developing methods which are mild, effective, cost-intensive and environment-friendly. These include physical, chemical, biological and combined approaches. The pretreatment methods are the increase the porosity of biomass particles and to increase the accessibility of cellulose and other polysaccharides to enzymes. The solublization is presumably associated with two types of chemical reactions: (a) the hydrolysis of xylans to sugars and oligosaccharides with much higher solubility than intact xylans and (b) the hydrolysis of lignin-xylan or xylan-xylan esters and of acetyl groups on polysaccharides[35]. Besides, additional strategies to improve the quality of subsequent enzyme hydrolysis and fermentation have been applied, which include multi-enzyme action, non-catalytic additives, high solids operation, multi-microbial systems, strain improvement, simultaneous pretreatment and saccharification, and efficient design of bioreactors[26].

Next generation bio-fuels shall involve technical components

- Biological sciences: Plant biotechnology, Cellular and molecular biology, microbial /industrial biotechnology.
- Chemical technology sciences: catalysis, reaction engineering and separations
- Feeding strategies: The enzymatic hydrolysis limitations could be overcome by feeding strategies as suggested by Koppram *et al*.[25] in his recent review. One strategy is with the enzymes either present in the reactor from start-up or fed into the reactor together with the substrate. This has shown beneficial effects on ethanol yield in fed-batch SSF of spruce at high dry matter[41]. In another study, using corn cobs as raw material, the gradual feeding of acid/alkali pretreated corn cob up to 25 % w/v dry matter ensured high hydrolysis yields, corresponding to a 15–20 % increase compared to batch processes with similar enzyme loadings[36].

Bio refineries

Nowadays, there is little commercial production of ethanol and ethanol derivatives from cellulosic biomass, but R and D is ongoing not only in Canada and USA, but also in Europe. For instance, in addition to the current 200 bio refineries operating in the USA in 2009, over the last year at least 28 advanced biofuel companies have started or planned cellulosic ethanol plants[42].

DISCUSSION

Bio fuels offer one of the best alternative options as they have much lower life cycle Green House Gas emissions compared to fossil fuels. These are liquid fuels derived from renewable biological sources[5,43-45]. One of the directives of European Union (2009/28/CE) imposes a quota of 10 % for bio fuels on all traffic fuel until 2020[48]. Although liquid biofuels are currently made almost entirely from sugar, starch or fats and oils, we believe that the use of food for fuel is not sustainable in the face of expanding demand for food, feed, and fiber and that the long-term opportunity to produce fuels from biomass will be largely restricted to using lignocelluloses and possibly algal lipids or terpenes. Ligno cellulosic feed stocks such as forest and agricultural residues, switch grass, woody plants and mixtures of prairie grasses, biomass from *Calotropis procera*, and other desert plants have been proposed to offer energy and environmental and economic advantages over current biofuel sources, because these feed stocks require limited or almost no agricultural inputs than annual crops[23,49-51]. Recent biotechnological advances made it possible to utilize biomass as a source for fuel molecules which can be divided into two phases: carbon chain elongation and functional modification. In addition to natural fatty acid and isoprenoid chain elongation pathways, keto acid-based chain elongation followed by decarboxylation and reduction has been explored for higher alcohol production. Second-generation bio fuel production from ligno cellulosic feed stocks (e.g., waste biomass and municipal solid waste) has been suggested to satisfy future EEC requirement for biofuels[25,52,53] Ligno cellulosic materials are regarded as good candidates for the second-generation ethanol production[48,49] even if cellulose, being embedded with hemicelluloses and lignin in the plant cell wall, has a partial crystalline structure and low accessibility. The ligno cellulosic ethanol production process has been a widely researched area in order to understand the bottlenecks that exist at each of the process steps and a significant progress has been made to overcome the challenges[28,54] Thus, adequate, but costly pretreatments are needed to enable its saccharification[29]. Bioenergy can positively contribute to climate goals and rural livelihoods; however, if not implemented carefully, it could exacerbate degradation of land, water bodies and ecosystems; reduce food security; and increase greenhouse gas (GHG) emissions. For large-scale commercial biofuels to contribute to sustainable development will require agriculturally sustainable methods and markets that provide enhanced livelihood opportunities and equitable terms of trade. The challenge lies in translating the opportunity into reality.

CONCLUSION

Although biofuel production is intrinsically an engineering problem, new developments in molecular biology, metabolic engineering, and systems biology enable wider choice of possible fuel molecules and production platforms. Ligno cellulosic biomass requires pretreatment. The pretreatment methods vary a great deal based on the feedstock used and hence, no single best method can be concluded. To enhance the production capacity of these pathways, metabolic engineering and protein engineering have been applied to (a) seek the best combination of genes from a variety of organisms to compose pathways in user-friendly hosts, (b) fine-tune the activity of different genes within the synthetic pathways, and (c) tailor individual enzymes for higher efficiency or novel catalytic ability. In summary, biofuel production with its interdisciplinary nature represents great challenges and opportunities for chemical and bio molecular engineers. The rapidly advancing tools will pave the way for biofuel to become a significant solution to energy and environmental problems. During production of this article, isobutanol production directly from CO_2 was achieved using photosynthetic bacteria[55].

REFERENCES

1. Harris PJ, Stone BA. Chemistry and molecular organization of plant cell walls. In Biomass Recalcitrance, ed. ME Himmel. Oxford: Blackwell; 2008. p. 60–93.
2. Kumar A. Economics of bioenergy in developing countries. In: Bioenergy 84 Vol.4, Bioenergy in developing countries. eds. Egneus H and Ellegard A (London + Elsevier Applied Science Publishers); 1984. p. 172.
3. Kumar A. Bioengineering of crops for bio fuels and bio energy. In: From soil to cell: A broad approach to plant life. Eds. Bender L and Kumar A. (Giessen + Electron. Library GEB); 2001. p. 1-16.
4. Kumar A. Bioengineering of crops for bio fuels and bio energy. In: Recent Advances in Plant Biotechnology. Eds. Kumar A and Sopory S (New Delhi + I K International); 2008. p. 346-360.
5. Cécile B, Fabien F, Benoît G and Bruno M. Bio fuels, greenhouse gases and climate change. Agron Sustain Dev 2011; 31: 1-79. http://dx.doi.org/10.1051/agro/2009039
6. Kumar A. Biofuel resources for Green House Gas Mitigation and Environment Protection. In: Agriculture Biotechnology, Ed. Trivedi PC. (Jaipur +Avishkar Publishers); 2011. p. 221-246.
7. Xavier AMRB, MF Correia, SR Pereira and DV Evtuguin. Second-generation bio ethanol from eucalypt sulphite spent liquor. Bio resource Technology 2010; 101: 1873-2976. http://dx.doi.org/10.1016 /j.biortech.2009.11.092
8. Mussatto SI, G Dragone, PMR Guimaraes, JPA Silva, LM Carneiro, IC Roberto, A Vicente, L Domingues and JA Teixeira. Technological trends, global market, and challenges of bio-ethanol production. Biotechnology Advances 2010; 28: 1873-1899. http://dx.doi.org/ 10.1016/j.biotechadv.2010.07.001
9. Kumar A. *Calotropis procera* (Ait). f. (Akra Sodom Apple) In: Agro technology Package for Bio energy crops. Eds. Swarup, R and Munshi M. (New Delhi + D.B.T. Govt. of India); 2007. p. 24-26.
10. Kumar A. Bio fuels Utilization: An Attempt to Reduce GHG's and Mitigate Climate Change. In: Knowledge Systems of Societies for Adaptation and Mitigation of Impacts of Climate change. (eds) Nautiyal S, Kaechele H, Rao KS, Schaldach R Springer-Verlag, Heidelberg, Germany; 2013. p. 199-224.
11. Santi G, Annibale AD, Eshel A Zilberstein, A Crognale M, Ruzzi Valentini R, Moresi M, Petruccioli M. Ethanol production from xerophilic and salt resistant *Tamarix jordanis* biomass. Bio mass and bio energy 2014; 6: 173-81.
12. Corwin DL, Lesch SM, Oster JD, Kaffka SR. Short-term sustainability of drainage water reuse: Spatio-temporal impact on soil chemical properties. J Env Qual 2008; 37: S8–24. http://dx.doi.org/ 10.2134/jeq2007.0140
13. Eshel A, Oren I, Alekperov C, Eilam T, Zilberstein A. Biomass production by desert halophytes: alleviating the pressure on the scarce resources of arable soil and fresh water. Eur J Plant Sci Bio technol 2011; 5(2): 48-53.
14. Schmer MR, Vogel KP, Mitchell RB, Perrin RK. Net energy value of cellulosic ethanol from switch grass. PNAS (The Proceedings of the National Academy of Sciences) 2008; 105: 464–9. http://dx.doi.org /10.1073/pnas.0704767105
15. Guretzky JA, Biermacher JT, Cook BJ, Kering MK and Mosali J. Switch grass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. Plant and Soil 2010; 339(1-2): 69–81. http://dx.doi.org/10.1007/s11104-010-0376-4
16. Ralph J, Guillaumie S, Grabber JH, Lapierre C, Barriere Y. Genetic and molecular basis of grass cell-wall biosynthesis and degradability. III. Towards a forage grass ideotype. C. R. Biol 2004; 327: 467–79. http://dx.doi.org/10.1016/j.crvi.2004.03.004

17. Carroll A and Somerville C. Cellulosic biofuels. Annual review of plant biology 2009; 60: 165–82. http://dx.doi.org/10.1146/annurev.arplant.043008.092125
18. Heaton EA, Flavell RB, Mascia PN, Thomas SR, Dohleman FG, Long SP. Herbaceous energy crop development: recent progress and future prospects. Curr. Opin. Biotechnol 2008; 19: 202–209. http://dx.doi.org/10.1016/j.copbio.2008.05.001
19. Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, *et al*. How biotech can transform biofuels. Nat. Biotechnol 2008; 26: 169–72. http://dx.doi.org/10.1038/nbt0208-169
20. Sims REH, Hastings A, Schlamadinger B, Taylor G, Smith P. Energy crops: current status and future prospects. Glob. Change Biol 2006; 12: 2054–76. http://dx.doi.org/10.1111/j.1365-2486.2006.01163.x
21. Sticklen MB. Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. Nat. Rev. Genet 2008; 9: 433–43. http://dx.doi.org/10.1038/nrg2336
22. Karp A, Shield I. Bioenergy from plants and the sustainable yield challenge. New Phytol 2008; 179: 15–32. http://dx.doi.org/10.1111/j.1469-8137.2008.02432.x
23. Kumar Ashwani. Bio fuels Utilization: An Attempt to Reduce GHG's and Mitigate Climate Change. In: Knowledge Systems of Societies for Adaptation and Mitigation of Impacts of Climate change. (eds) Nautiyal S, Kaechele H, Rao KS, Schaldach R Springer-Verlag, Heidelberg, Germany; 2013. p. 199-224.
24. Koppram R, Tomás Pejó E, Xiros C and Olsson L. Ligno cellulosic ethanol production at high-gravity: challenges and perspectives. Trends in Biotechnology 2014; 32(1): 46–53. http://dx.doi.org/10.1016/j.tibtech.2013.10.003
25. Sims RE, Mabee W, Saddler JN, Taylor M. An overview of second generation biofuel technologies. Bio resour Technol 2010; 101(6): 1570-80. http://dx.doi.org/10.1016/j.biortech.2009.11.046
26. Roy Anindita and Kumar A. Pretreatment Methods of Ligno cellulosic Materials for Bio fuel Production: A Review. Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 2013; 4(2): 181-193.
27. Galbe M, Zacchi G. A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 2002; 59(6): 618-628. http://dx.doi.org/10.1007/s00253-002-1058-9
28. Alvira P *et al*. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour. Technol 2010; 101: 4851–4861. http://dx.doi.org/10.1016/j.biortech.2009.11.093
29. Chen H and Qiu W. Key technologies for bioethanol production from lignocellulose. Biotechnol Adv 2010; 28(5): 556-62. http://dx.doi.org/10.1016/j.biotechadv.2010.05.005
30. Kumar A. Biofuel resources for Green House Gas Mitigation and Environment Protection. In: Agriculture Biotechnology, Ed. Trivedi PC. (Jaipur + Avishkar Publishers); 2011. p. 221-246.
31. Pereira FB *et al*. Identification of candidate genes for yeast engineering to improve bioethanol production in very high gravity and ligno cellulosic biomass industrial fermentations. Biotechnol. Bio fuels 2011; 4: 57.
32. Fischer CR, Klein Marcuschamer D, Stephanopoulos G. Selection and optimization of microbial hosts for biofuels production. Metab. Eng 2008; 10: 295–304. http://dx.doi.org/10.1016/j.ymben.2008.06.009
33. Ho NW, Chen Z, Brainard AP. Genetically engineered *Saccharomyces* yeast capable of effective co fermentation of glucose and xylose. Appl. Environ. Microbiol 1998; 64: 1852–59.
34. Kuyper M, Toirkens MJ, Diderich JA, Winkler AA, Van Dijken JP, Pronk JT. Evolutionary engineering of mixed-sugar utilization by a xylose-fermenting *Saccharomyces cerevisiae* strain. FEMS Yeast Res 2005; 5: 925–34. http://dx.doi.org/10.1016/j.femsyr.2005.04.004
35. Van Maris AJ, Winkler AA, Kuyper M, De Laat WT, Van Dijken JP, Pronk JT. Development of efficient xylose fermentation in *Saccharomyces cerevisiae*: xylose isomerase as a key component. Adv. Biochem. Eng. Biotechnol 2007; 108: 179–204. http://dx.doi.org/10.1007/10_2007_057
36. Zhang MJ, Wang F, Su RX *et al*. Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. Bioresour Technol 2010; 101(13): 4959—4964. http://dx.doi.org/10.1016/j.biortech.2009.11.010
37. Lu Y *et al*. Improvement of robustness and ethanol production of ethanologenic *Saccharomyces cerevisiae* under co-stress of heat and inhibitors. J. Ind. Microbiol. Biotechnol 2012; 39: 73–80. http://dx.doi.org/10.1007/s10295-011-1001-0
38. Endo A, Nakamura T and Shima J. Involvement of ergosterol in tolerance to vanillin, a potential inhibitor of bioethanol fermentation, in *Saccharomyces cerevisiae*. FEMS Microbiology Letters 2009; 299(1): 95–9. http://dx.doi.org/10.1111/j.1574-6968.2009.01733.x
39. Hasunuma T, Sanda T, Yamada R, Yoshimura K, Ishii J and Kondo A. Metabolic pathway engineering based on metabolomics confers acetic and formic acid tolerance to a recombinant xylose-fermenting strain of *Saccharomyces cerevisiae*. Microbial Cell Factories 2011; 10(1): 2-13. http://dx.doi.org/10.1186/1475-2859-10-2
40. Jarboe LR, Grabar TB, Yomano LP, Shanmugan KT, Ingram LO. Development of ethanologenic bacteria. Adv. Biochem. Eng. Biotechnol 2007; 108: 237–61. http://dx.doi.org/10.1007/10_2007_068
41. Hoyer K, Galbe M and Zacchi G. Effects of enzyme feeding strategy on ethanol yield in fed-batch simultaneous saccharification and fermentation of spruce at high dry matter 2010; 3: 2-11.
42. Ondrey G. Bio alcoholic fuels. Chem. Eng 2010; 117 (5): 25–29.
43. El Bassam N. Energy Plant Species: Their Use and Impact on Environment and Development. London: James and James; 1998. p. 321.
44. Kumar A. Bioengineering of crops for biofuels and bioenergy. In: From soil to cell: A broad approach to plant life. Eds. Bender L and Kumar A. (Giessen + Electron. Library GEB); 2001. p. 1-16.
45. Kumar A. Bioengineering of crops for biofuels and bioenergy. In: Recent Advances in Plant Biotechnology. Eds. Kumar A and Sopory S (New Delhi + I K International); 2008. p. 346-360.
46. Kumar A. Biofuel resources for Green House Gas Mitigation and Environment Protection. In: Agriculture Biotechnology, Ed. Trivedi PC. (Jaipur + Avishkar Publishers); 2011. p. 221-246.
47. Rana A and Kumar A. Studies on production of hydrocarbons from *Calotropis procera* a bio fuel plant. Germany Lambert Academic Publishers; 2012. p. 220.
48. Xavier MR. The Brazilian sugarcane ethanol experience, Competitive Enterprise Institute CEI Issue Analysis, Advancing Liberty from the Economy to Ecology 2007; 3: 13.
49. Kumar A and Kumar VR. Bioenergy potential of semi-arid regions of Rajasthan. In: Biomass for energy, Industry and climatic protection, Eds. Palz W, Spitzer J, Maniatis K, Kwant K, Helm P and Grassi A. (Germany + ETA-Florence and WIP Munich); 2002. p. 372-374.
50. Kumar VR, Kumar A and Gupta AK. *Calotropis procera*: A potential bio-energy plant for arid and semi-arid regions. In Biomass for energy, Industry and climatic protection. Eds. Palz W, Spitzer J, Maniatis K, Kwant K, Helm P and Grassi A (Germany + ETA-Florence and WIP Munich); 2002. p. 375-377.
51. Kumar A. *Calotropis procera* (Ait). f. (Akra Sodom Apple) In: Agro technology Package for Bio energy crops. Eds. Swarup R and Munshi M. (New Delhi + D.B.T. Govt. of India); 2007. p. 24-26.
52. Hahn Hagerdal B, Galbe M, Gorwa Grauslund MF, Liden G, Zacchi G. Bio-ethanol-the fuel of tomorrow from the residues of today. Trends Biotechnol 2006; 24(12): 549-56. http://dx.doi.org/10.1016/j.tibtech.2006.10.004
53. Directive 2003/30/EC of the European Parliament of the Council of on the promotion of the use of biofuels or other renewable energy. Official Journal of European Union 17.05.2003. L123, 42; 2003.
54. Lynd LR *et al*. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev 2002; 66: 506–577. http://dx.doi.org/10.1128/MMBR.66.3.506-577.2002
55. Atsumi S, Higashide W, Liao JC. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat. Biotechnol 2009; 27: 1177–80. http://dx.doi.org/10.1038/nbt.1586
56. Schubert C. Can biofuels finally take center stage? Nature Biotechnology 2006; 24: 777-784. http://dx.doi.org/10.1038/nbt0706-777

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