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Fermentation of Biomass for Production of Ethanol: A Review

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Abstract:

The Present world energy scenario is focused at nonconventional sources. The biomass has emerged as one of the dependable nontraditional feed stocks for the production ethanol. The present review enlighten various feed stocks viz. sugar beets, sugar cane corn, wheat, barley etc. and fermentation methods for the production of ethanol. The use of biomass for clean energy generation in the European Union is expected to increase nearly 35% by the end of 2030 without harming biodiversity, soil and water resources. Ethanol can be produced from appreciable amount of sugar or material that can be converted into sugar such as starch or cellulose. Industrial ethanol producing microorganisms which are capable of fermenting all of the sugar present in feed stock; attracted much attention in recent years with the recent advances in biotechnology. Saccharomyces cerevisiae (yeast) is naturally unable to ferment pentose; its capability for xylose utilization has successfully been improved by intensive research over the last decades. During the last fifteen years, research has been focused on finding these xylose-fermenting microorganisms. S. cerevisiae has an efficient anaerobic sugar metabolism, tolerates inhibitory industrial substrates better than other microorganisms and ferments hexoses abundantly present in lignocellulosic hydro lysates with high yield and productivity. Attempts have also been made to review the status of fermentation of forest, industrial residue, agriculture waste and municipal solid waste. The present efforts are expected to enhance world energy scenario and life on the planet.

Keywords: Biomass, Ethanol, Fermentation, Saccharomyces cerevisiae.

1. Introduction:

Ethanol is one of the best tools to fight vehicular pollution, contains 35% oxygen that helps complete combustion of fuel and thus reduces harmful tailpipe emissions. It also reduces particulate emissions that pose a health hazard. The petroleum industry looks very committed to the use of ethanol as fuel, as it is expected to benefit sugarcane farmers as well as the oil industry in the long run. Ethanol can also be produced from wheat, corn, beet, sweet sorghum etc. In January 2003, the government of India launched a program to mandate the blending of 5% ethanol in gasoline. In the first phase of the project, ethanol- blended petrol is being supplied through retail outlets in nine States and four Union Territories. These states are Andhra Pradesh, Goa, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh. The four Union Territories include Chandigarh, Dadra and Nagar Haveli, Daman and Diu and Pondicherry. Petrol blended with 5 per cent ethanol would be supplied by petrol pumps all over the country under the second phase towards the end of the year. The content of ethanol blending would be increased to 10 per cent in the third phase of the program scheduled for 2005. Table-1 shows demand of gasoline and diesel in India.

Table 1: Demand of gasoline and diesel (Million tons) in India

Year	Gasoline	Diesel
2002–2003	7.62	42.15
2003–2004	8.20	44.51
2004–2005	8.81	46.97
2005–2006	9.42	49.56
2006–2007	10.07	52.33
2011–2012	12.85	66.90

(Source: Planning and Commission, Government of India. 2011)

Ethanol, being an excellent transportation fuel can be used as blend with gasoline, 10 and 22% blends are being used in the US and Brazil, respectively (Wyman [41], Wei-Dong[38]). It is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NOx emission from combustion. It may be used directly (95% ethanol and 5% water) as a fuel, such nearly pure ethanol fuel provides a number of environment benefits, due to their low pressure and reduced emission of ethanol in to the atmosphere along with their clean burning characteristics. Since ethanol is produced from plants that harness the power of the sun, ethanol is also considered a renewable fuel. Therefore, ethanol has many advantages as an automotive fuel.

2. Potential of Biomass

The continuous growth of plants on our planet exceeds men's primary energy requirements many times over. Of course, only part of the biomass that grows can actually be supplied for energy use, due to ecological, technical and economic reasons. However, there remains a huge amount of biomass that is very suitable for exploitation. Biomass resources comprise those which are received from agriculture and forestry as well as from agro- and wood industries. It also includes waste sources from construction and demolition as well as municipal wastes. According to the European Environment Agency (EEA), the use of biomass for clean energy generation in the European Union could be significantly increased in the next decades without harming biodiversity, soil and water resources. The potential biomass available in Europe seems to be sufficient to support the ambitious renewable energy targets in an environmentally responsible way. Extracted from agriculture, forestry and organic waste, biomass can provide heat, power and transport fuels in an environmentally friendly way.

Nibedita et al (2012) [18] classified four major agro wastes as the most favorable feed stocks for bioethanol production due to their availability throughout the year. Worldwide production of these agro wastes is given in Table 2. Asia is the major producer of rice straw and wheat straw, whereas corn straw and bagasse are mostly produced in America. Lignocellulosic materials are renewable, low cost and are abundantly available. It includes crop residues, grasses, sawdust, wood chips, etc. Extensive research has been carried out on ethanol production from lignocellulosic in the past two decades.

Table 2: Quantities of agricultural waste (Million tons) available for bioethanol production.

Agro waste	**Africa**	**Asia**	**Europe**	**America**	**Oceania**
Rice straw	20.9	667.6	3.9	37.2	1.7
Wheat straw	5.34	145.20	132.59	62.64	8.57
Corn straw	0.00	33.90	28.61	140.86	0.24
Bagasse	11.73	74.88	0.01	87.62	6.49

(Source: Nibedita et al, 2012)

The European Environment Agency has recently assessed the quantity of the potential European "environmentally-compatible biomass" and shows that the potential of environmentally compatible biomass for producing energy could increase from the predicted 190 M tones in 2010 to about 295 M tones in 2030. Considering only the potential of environmentally-compatible agricultural bioenergy and excluding the bioenergy potential from forestry and from wastes, the EEA assessed that around 47 M tones of bioenergy can be derived from the released agricultural land area in 2010 without creating additional environmental pressures. This production could increase to around 95 M tones in 2020 and 144 M tones in 2030 as shown in Figure A. (Bio Fuel Technology Handbook, WIP 2007)

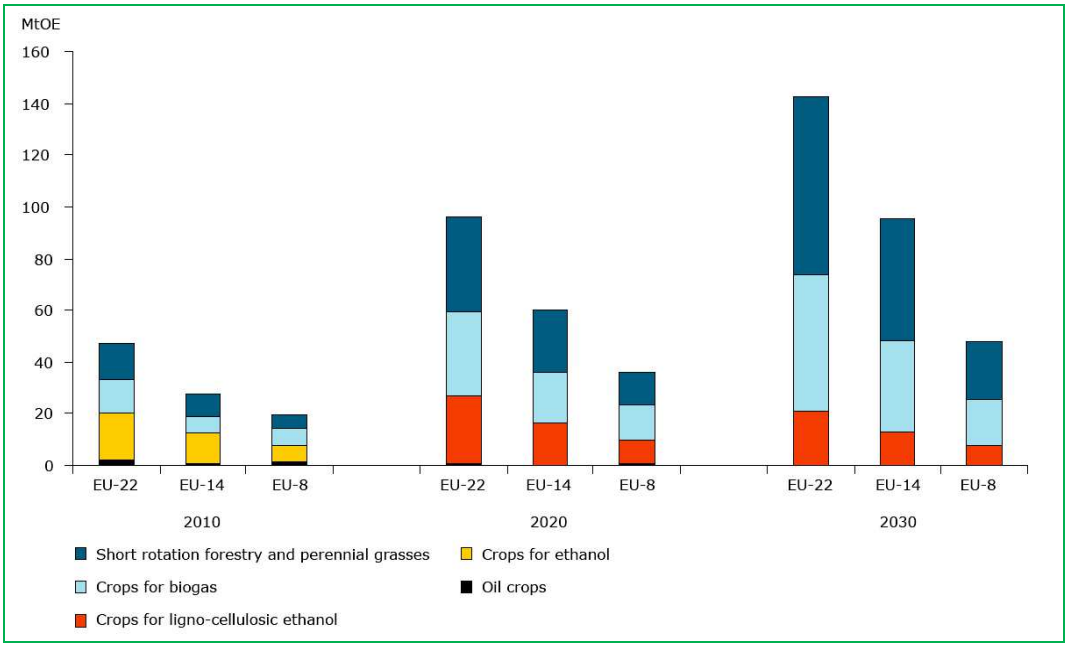

Figure A: Environmentally-compatible agricultural bioenergy potential
(Source: Biofuel Technology Handbook, WIP 2007)

3. Feed stocks

Feed stocks can be derived from agricultural, forest, or municipal waste products. Biomass can come in almost any form. Typical feed stocks include forest residues, agricultural crops and residues, wood and wood residues, aquatic plants, and fast-growing trees and plants. Typical waste feed stocks include municipal solid waste, construction waste, agricultural waste, animal manure, meatpacking waste, food processing waste, spent pulping liquor, waste cooking oil, paper mill residue and wastewater treatment sludge. The process through which the biomass is converted to energy is dependent on the chemical composition, homogeneity, size, amount, and water content of the feedstock. Also, geographic location is a consideration, as biomass must be transported from farm to bio-refinery. (Biomass Conversion, EPA 2007). Ethanol can be produced from any biological feedstock that contains appreciable amounts of sugar or materials that can be converted into sugar such as starch or cellulose. Many different feedstock sources can be used for ethanol production as shown in Figure B. Two examples of feedstock for ethanol production are sugar beets and sugar cane which contain high percentages of sugar. Sugars can be easily fermented. Corn, wheat, barley, rye and other cereals are typical feedstock containing starch in their kernels. Starch can relatively easily be converted into sugar and then into ethanol. In the USA and Europe, ethanol is manufactured mainly from maize and grain. (Biofuels for Transportation, Global Potential and Implications for Sustainable Agriculture and Energy in the 21st Century, WWI 2006)

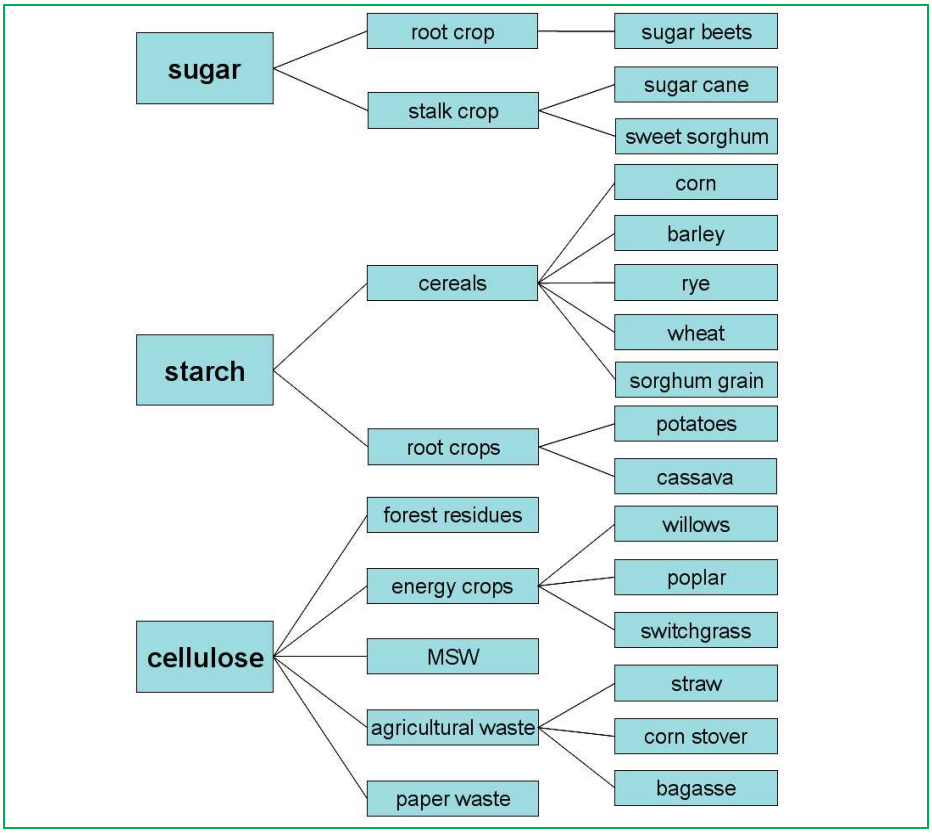

Figure B: Types of feedstock for ethanol production with examples. (Source: WWI, 2006)

4. Microorganism involve in ethanol production

Microorganisms have long played a major role in the production of food (dairy, fish and meat products) and alcoholic beverages. In addition, several products of microbial fermentation are also incorporated into food as additives and supplements (antioxidants, flavors, colorants, preservatives, sweeteners, etc.). There is great interest in the development and use of natural food and additives derived from microorganisms, since they are more desirable than the synthetic ones produced by chemical processes. Solid-state fermentation (SSF) reproduces the natural microbiological processes like composting and ensiling. In industrial applications this natural process can be utilized in a controlled way to produce a desired product. Typical examples of it are the fermentation of rice by Aspergillus oryzae to initiate the koji process and Penicillium roquefortii for cheese production. (Susana et al)[32]

SSF is defined as any fermentation process performed on a non-soluble material that acts both as physical support and source of nutrients in absence of free flowing liquid. The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used. Some examples of SSF processes for each category of microorganisms are reported in Table 3.

Table 3: Main groups of microorganisms involved in SSF processes

Microflora	**SSF process**
Bacteria	
Bacillus sp.	Composting, natto, amylase
Pseudomonas sp.	Composting
Serratia sp.	Composting
Streptococcus sp.	Composting
Lactobacillus sp.	Ensiling, food
Clostridium sp.	Ensiling, food
Yeast	
Endomicopsis burtonii	Tape cassava, rice

Saccharomyces cerevisiae	Food, ethanol
Schwanniomyces castelli	Ethanol, amylase
Fungi	
Altemaria sp.	Composting
Aspergillus sp.	Composting, industrial, food
Fusarium sp.	Composting, gibberellins
Monilia sp.	Composting
Mucor sp.	Composting, food, enzyme
Rhizopus sp.	Composting, food, enzymes, organic acids
Phanerochaete chrysosporium	Composting, lignin degradation
Trichoderma sp.	Composting, biological control, bio insecticide
Beauveria sp., Metharizium sp.	Biological control, bio insecticide
Rhizopus oligosporus	Tempeh, soybean, amylase, lipase
Aspergillus niger	Feed, proteins, amylase, citric acid
Pleurotus oestreatus, sajor-caju	Mushroom
Lentinus edodes	Shii-take mushroom
Penicilium notatum, roquefortii	Penicillin, cheese

(Source: Susana R. C. et al, 2006)

Industrial process for ethanol production from lignocelluloses requires that the yeast is capable of fermenting all of the sugars present with high ethanol yields and productivities. Although Saccharomyces cerevisiae is naturally unable to ferment pentoses, its capability for xylose utilization has successfully been improved by intensive research over the last decades. A key aspect of metabolic engineering in yeast has been the heterologous expression of genes for xylose reductase (XR) and xylitol dehydrogenase (XDH) derived from Pichia stipitis, combined with overexpression of S. cerevisiae xylulokinase (XK); in combination, these enzymes are responsible for the initial steps of xylose assimilation. Lisbeth and Barbel (1996) [13] indicate efficient xylose fermenting microorganisms have been found among bacteria, yeasts, and fungi (natural as well as recombinant). During the last fifteen years, research has been focused on finding these xylose-fermenting microorganisms and understanding the xylose metabolism while less research has been concerned with the arabinose metabolism. Typical ethanol yields and total volumetric ethanol productivities for batch fermentations with these microorganisms in laboratory medium using xylose as the carbon source are summarized in Table 4.

Table 4: Performance of xylose-fermenting microorganisms

Strain	Xylose	Ethanol	Yield
Bacteria: naturally occurring			
Bacillus macerans DMS 1574	20	3.3	0.16
Bacteroides polypragmatus NRCC 2288	44	6.5	0.15
Clostridium saccharolyticum ATCC 35040	25	5.2	0.21
Bacteria: recombinant			
Escherichia coil B, pLOI297	80	39.2	0.49
E. coil B KO11	80	41.6	0.52
Klebsiella oxytoca M5A1	100	46.0	0.46
Zymomonas mobilis CP4	25	11.0	0.44
Yeasts: naturally occurring			
C. shehatae CBS 4705	50	24.0	0.48
C. shehatae CSIR-Y492	90	26.2	0.29
P. tannophilus RL 171	50	13.8	0.28
P. stipitis CBS 5776	50	22.3	0.45
Yeasts: recombinant			
Saccharomyces cerevisiae (XYL 1, XYL 2)	21.7	1.60	0.07
Schizosaccharomyces pombe	50	21.0	0.42
Fungi			
Fusarium avenaceum VTT-D-80146	50	12.0	0.24
F. graminearum VTT-D-79129	50	11.0	0.22
F. lycopersici ATCC 16417	50	16.0	0.32
F. oxysporum VTT-D-80134	50	25.0	0.50

(Lisbeth et al, 1996)

Genetically engineered microorganisms that can convert xylose and/or pentose to ethanol can greatly improve ethanol production efficiency and reduce the cost of production. The constructed operons encoding xylose assimilation and pentose phosphate pathway enzymes were transformed into the bacterium Zymomonas mobilis for the effective fermentation of xylose to produce ethanol. The recombinant plasmids with xylose reductase (XR) and xylitol dehydrogenase (XDH) genes from Pichia stipitis and xylulokinase (XK) gene from S. cerevisiae have been transformed into Saccharomyces spp. for the co-fermentation of glucose and xylose. Prasad et al (2007)[24] Ethanol-producing bacteria have attracted much attention in recent years because their growth rate is substantially higher than that of the Saccharomyces presently used for practical production of fuel alcohol and, with the recent advances in biotechnology, they have the potential to play a key role in producing ethanol more economical. Among such ethanol-producing bacteria, Z. mobilis is a well-known organism used historically in tropical areas to make alcoholic beverages from plant sap. The advantages of Z. mobilis are its high growth rate and specific ethanol production; unfortunately, its fermentable carbohydrates are limited to glucose, fructose and sucrose. On the other hand, the Gram-negative strain Zymobacter palmae, which was isolated by Okamoto et al. (1993) [20] using a broad range of carbohydrate substrates, is a facultative anaerobe that ferments hexoses, -linked di- and tri-saccharides, and sugar alcohols (fructose, galactose, glucose, mannose, maltose, melibiose, sucrose, raffinose, mannitol and sorbitol). This strain produces approximately 2 mol of ethanol per mole of glucose without accumulation of byproducts and shows productivity similar to that of Z. mobilis. Numerous studies have addressed the challenges of breeding of alcohol-producing microorganisms that harbor a pet operon, including E. coli, E. chrysanthemi and Klebsiella oxitoca, which can produce ethanol from cellulosic materials. So far, however, the production of ethanol from cellulosic materials using these strains has not reached a level sufficient for commercial application. S. cerevisiae has an efficient anaerobic sugar metabolism, tolerates inhibitory industrial substrates better than other microorganisms and ferments hexoses abundantly present in lignocellulosic hydrolysates, such as glucose, mannose and galactose with high yield and productivity. With genetically stable strains, the application of a fermentation technique such as fed-batch or continuous fermentation that facilitates the simultaneous fermentation of xylose and other sugars may solve these problems. [24]

5. Fermentation of forest and industrial residues

Raw materials containing sugars, or materials which can be transformed into sugars, can be used as fermentation substrates. The fermentable raw materials can be grouped as directly fermentable sugary materials, starchy, lignocellulosic materials and urban/industrial wastes. Direct fermentation of sugarcane, sugar beet and sweet sorghum to produce ethanol has also been reported (Bryan, 1990; Ganesh et al., 1995; Ravi et al., 1997)[6][10][27]. Sugar containing materials require the least costly pretreatment, where starchy, lignocellulosic materials and urban/industrial wastes needed costly pretreatment, to convert into fermentable substrates (Sun and Cheng, 2002) [31]. Sugar containing materials which can be transformed into glucose, can be used as fermentation substrates under anaerobic conditions, glucose is converted to ethanol and carbon dioxide by glycolysis.

Rice straw is one of the most abundant lignocellulosic feedstock in the world. In Asia, it is produced about 667.6 million tons annually. Wen-Heng et al (2012) [39] used rice straw as raw material to produce ethanol with single fermentable strain, P. sitpitis has been studied. Without any nutritional supplementation, the ethanol yield were around 0.45~0.5 g/g in the rice straw hydrolysates. Particularly, the high ethanol yield form rice straw hydro lysate mixture by P. stipits showed the potential of developing the co-fermentation process. The lower constitutes of lignin and acetyl group in the rice straw is reflects the less inhibition during hydrolysate fermentation. Overliming employed was also effective in removing phenolic compounds. These results revealed a promising perspective to develop more attractive ethanol production process from rice straw as raw material using P.stipitis.

Anuchit et al (2011)[2] proposed thin-shell silk cocoon (TSC), a residual from the silk industry, which is used as a support material for the immobilization of Saccharomyces cerevisiae in ethanol fermentation because of its properties such as high mechanical strength, light weight, biocompatibility and high surface area. In batch fermentation with blackstrap molasses as the main

fermentation substrate, an optimal ethanol concentration of 98.6 g/L was obtained using a TSC-immobilized cell system at an initial reducing sugar concentration of 240 g/L. The ethanol concentration produced by the immobilized cells was 11.5% higher than that produced by the free cells. Ethanol production in five-cycle repeated batch fermentation demonstrated the enhanced stability of the immobilized yeast cells. Under continuous fermentation in a packed-bed reactor, a maximum ethanol productivity of 19.0 g/(L h) with an ethanol concentration of 52.8 g/L was observed at a 0.36 h-1 dilution rate. Farshid et al (2011) [9] studied the continuous fermentation of cane molasses in an immobilized cells reactor. Sodium-alginate immobilized yeast was employed to produce ethanol continuously using cane molasses as a carbon source in an immobilized cell reactor (ICR). The immobilization of Saccharomyces cerevisiae was performed by entrapment of the cell cultured media harvested at exponential growth phase (16 h) with 3% sodium alginate. During the initial stage of operation, the ICR was loaded with fresh beads of mean diameter of 5.01 mm. The ethanol production was affected by the concentration of the cane molasses (50, 100 and 150 g/l), dilution rates (0.064, 0.096, 0.144 and 0.192 h–1) and hydraulic retention time (5.21, 6.94, 10.42 and 15.63 h) of the media. The pH of the feed medium was set at 4.5 and the fermentation was carried out at an ambient temperature. The maximum ethanol production, theoretical yield, volumetric ethanol productivity and total sugar consumption was 19.15 g/l, 46.23%, 2.39 g/l/h and 96%, respectively.

Nancy et al (2010) [17] investigated biological abatement for removal of inhibitors. Biological abatement was used to condition dilute acid-pretreated hydro lysates of three perennial herbaceous crops that are potential bioenergy feed stocks: switch grass, reed canary grass, and alfalfa stems. Fungal isolate Coniochaeta ligniaria was inoculated into the hydro lysates to metabolize and remove inhibitory compounds prior to yeast fermentation of glucose. Switch grass, reed canary grass, and alfalfa stem samples were pretreated with dilute acid at 10% w/w biomass loading and subjected to bio abatement with strain NRRL30616, to prepare the material for simultaneous saccharification of cellulose and fermentation by Saccharomyces cerevisiae. Bio abatement eliminated the extended fermentation lag times associated with inhibitory compounds and observed for the unconditioned biomass hydro lysates controls. Bio abatement was as effective as lime conditioning in reducing fermentation lag times. Prolonged incubations with the bio abatement microbe resulted in consumption of some glucose and reduced production of ethanol.

Ramesh et al (2010) [25] studied lantana camara (red sage) contains 61.1% (w/w) holocellulose and can serve as a low-cost feedstock for bioethanol production. Acid hydrolysis (3.0%, v/v H2SO4, 120°C for 45 min) of L. camara produced 187.14 mg/g total sugars along with fermentation inhibitors such as phenolics (8.2 mg/g), furfurals (5.1 mg/g) and hydroxyl methyl furfurals (6.7 mg/g). Sequential application of over liming (pH 10.0) and activated charcoal (1.5%, w/v) adsorption was used to remove these toxic compounds from the acid hydrolysate. The acid-pretreated biomass of L. camara was further delignified through combined pretreatment of sodium sulphite (5.0% w/v) and sodium chlorite (3.0% w/v), which resulted in about 87.2% lignin removal. The enzymatic hydrolysis of delignified cellulosic substrate showed 80.0% saccharification after 28 h incubation at 50°C and pH 5.0. Fermentation of acid and enzymatic hydro lysates with Pichia stipitis and Saccharomyces cerevisiae gave rise to 5.16 and 17.7 g/L of ethanol with corresponding yields of 0.32 and 0.48 g/g after 24 and 16 h, respectively.

Sujit et al (2009) [29] found a new and cheap carbohydrate sources for production of bioethanol. In this context, the production of ethanol from mahula (Madhuca latifolia L.) flowers by Saccharomyces cerevisiae in solid-state fermentation. The moisture level of 70%, pH of 6.0 and temperature of 30°C were found optimum for maximum ethanol concentration (225.0 ± 4.0 g/kg flower) obtained from mahula flowers after 72 h of fermentation. Concomitant with highest ethanol concentration, the maximum ethanol productivity (3.13 g/kg flower/h), yeast biomass (18.5 x 108 CFU/g flower), the ethanol yield (58.44 g/100 g sugar consumed) and the fermentation efficiency (77.1%) were also obtained at these parametric levels. Swain et al (2007) [33] takes interest to find alternate bio resources for production of ethanol, apart from cane/sugar beet molasses and starchy crops like sweet sorghum, cassava and sweet potato. Mahula (Madhuca latifolia L.) is a forest tree abundantly available in the Indian subcontinent and its flowers are very rich in fermentable sugars (28.1–36.3 g 100 g-1). Batch fermentation of fresh and 12-month-stored flowers with free (whole cells) and immobilized cells of Saccharomyces cerevisiae was carried out

in 2-l Erlenmeyer flasks. The ethanol yields were 193 and 148 g kg-1 (using free cells) and 205 and 152 g kg-1 (using immobilized cells) from fresh and 12-month-stored mahula flowers, respectively.

6. Fermentation of agricultural wastes

Globally, bioethanol production from rice straw, wheat straw, corn straw and sugarcane bagasse is now a matter of interest as indicated in Table 5. Rice straw is the most abundant waste compared to the other major wastes and rice straw can potentially produce 205 billion liters bioethanol per year, which is the highest among these four mentioned agricultural wastes.

Table 5: Worldwide potential bioethanol production from agricultural wastes.

Agricultural residue	Potential annual bioethanol production (globally) (giga liter)
Rice straw	205
Wheat straw	104
Corn straw	58.6
Sugarcane bagasse	51.3

(Source: Nibedita et al, 2012)

Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. Conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed. Therefore, lignocellulosic substances such as agricultural wastes are attractive feed stocks for bioethanol production. Agricultural wastes are cost effective, renewable and abundant. Bioethanol from agricultural waste could be a promising technology though the process has several challenges and limitations such as biomass transport and handling, and efficient pretreatment methods for total delignification of lignocellulosic. Proper pretreatment methods can increase concentrations of fermentable sugars after enzymatic saccharification, thereby improving the efficiency of the whole process. Conversion of glucose as well as xylose to ethanol needs some new fermentation technologies, to make the whole process cost effective. In this review, available technologies for bioethanol production from agricultural wastes are discussed.

The most important processing challenge in the production of biofuel is pretreatment of the biomass. Lignocellulosic biomass is composed of three main constituents namely hemicellulose, lignin and cellulose. Pretreatment methods refer to the solubilization and separation of one or more of these components of biomass suggested. It makes the remaining solid biomass more accessible to further chemical or biological treatment. Goals of an effective pretreatment process are (i) formation of sugars directly or subsequently by hydrolysis (ii) to avoid loss and/or degradation of sugars formed (iii) to limit formation of inhibitory products (iv) to reduce energy demands and (v) to minimize costs. Physical, chemical, physicochemical and biological treatments are the four fundamental types of pretreatment techniques employed.

Nibedita et al (2012) [18] investigated saccharification is the critical step for bioethanol production where complex carbohydrates are converted to simple monomers. Compared to acid hydrolysis, enzymatic hydrolysis requires less energy and mild environment conditions. The optimum conditions for cellulase have been reported as temperature of 40-50°C and pH 4-5. Assay conditions for xylanase have also been reported to be 50°C temperature and pH 4-5. Therefore, enzymatic hydrolysis is advantageous because of its low toxicity, low utility cost and low corrosion compared to acid or alkaline hydrolysis. Moreover, no inhibitory by-product is formed in enzymatic hydrolysis. However, enzymatic hydrolysis is carried out by cellulase enzymes that are highly substrate specific. Finally, in case of fermentation configuration, the challenges involved are xylose and glucose co-fermentation, and the use of recombinant microbial strains. In conclusion it may be said that to solve the technology bottlenecks of the conversion process, novel science and efficient technology are to be applied, so that bioethanol production from agricultural wastes may be successfully developed and optimized in the near future.

Amrita et al (2011)[1] produced economically feasible cellulosic ethanol. Ethanol yield and enzyme efficiency has to be improved by optimizing all unit processes (pretreatments, saccharification and ethanol fermentation). Most of the pretreatments focused on low temperature pretreatment to avoid degradation of hemicellulose and to improve the pentose yield. Impregnation by various chemical reagents such as

sulfuric acid and application of microwave have been tried to lower pretreatment temperatures while maintaining high enzymatic digestibility. For saccharification, the accessible surface area and enzyme, reuses were key parameters. With regard to surface area, xylanases addition was effective. To prevent deactivation of cellulase by binding to non-productive sites, the addition of surfactants was the efficient method. Among various reagents, PEG 6000 exhibited best performance. Co-fermentation of glucose and xylose was key factor in improving ethanol yield. Fed-batch and co immobilization have been found to be the ideal option for co-fermentation steps.

Chi-Wen et al (2011) [8] constructed a mixed culture from compost of Napiergrass and sheep dung under anaerobic thermophilic conditions (60°C). The native microflora was cultivated for numerous generations to obtain a stable mixed culture that can degrade lignocelluloses. The fifth generation of the mixed culture consisting of five main bacteria (Clostridium strain TCW1, Bacillus sp. THLA0409, Klebsiella pneumoniae THLB0409, Klebsiella oxytoca THLC0409, and Brevibacillus strain AHPC8120) was employed to investigate the effects of operating conditions on culture growth and production of biochemical products, including ethanol. The mixed culture effectively degraded a diverse range of lignocellulosic materials, including microcrystalline cellulose (avicel) and natural lignocelluloses (Napiergrass). Acetic acid, ethanol, and butanol were the main biochemical products produced by biological fermentation. Under optimal conditions, ethanol yields from avicel and Napier grass reached maxima of 0.108 and 0.040 g g−1, representing ethanol productivities of 0.00055 and 0.00028 g g−1 h−1, respectively.

Mitchell et al (2010) [15] produced crude unprocessed cellulase extracts by solid-state fermentation of Trichoderma reesei on ground wheat straw. While cellulase yields were not high they were sufficient to produce ethanol from wheat straw in simultaneous saccharification and fermentation with Saccharomyces cerevisiae. As little as an additional 5% of the material converted to ethanol may be employed for cellulase production suggesting an inordinate quantity of additional substrate would not be required. These findings suggest a simplified crude cellulase process at the site of ethanol production using a common lignocellulosic substrate which employed in lieu of commercial enzyme preparations. A series of solid-state fermentations were carried out starting with 25 or 50 g dry ground wheat straw and 50 or 100 ml nutrient solution in 500 ml Schott bottles with cotton bungs and aluminum foil. Initially batch solid-state fermentations, inoculated with a common spore suspension, were run in duplicate for 10 and 14 days. Diluted crude cellulase extract obtained from solid-state fermentations was used to carry out the simultaneous saccharification and fermentation of ground wheat straw to ethanol. It was desired to see if the crude unprocessed cellulase could actually be used to produce ethanol, to get an indication of the levels of cellulase activity required and to see the effect of varying cellulase activity on the amount of ethanol produced. Two batch fermentations and one fed-batch fermentation were carried out.

Ying et al (2010) [42] proposed use of non-food crops for bioethanol production. The system consists of the following processes: sweet sorghum cultivation, crude ethanol production, and ethanol refining and by-product utilization. The plant capacities of crude ethanol and pure ethanol, in different fractions of useful land, are optimized. Assuming a minimum cost of investment, transport, operation and so on, the optimum capacity of the pure ethanol factory is 50,000 tones/year. Sweet sorghum is a non-food crop that will not occupy large areas of agricultural land. A large amount of gasoline could be substituted with sweet sorghum ethanol to ease constraints on the use of fossil fuels, reduce CO2 emissions and consequently, mitigate climate warming. An agro-industrial system for producing sweet sorghum bioethanol is described in this paper. In order to optimize the system, a two-tier nonlinear programming model was built. Based on the optimization results, when the fraction of useful land is 1.0, an optimum system is obtained with a pure ethanol factory capacity of 50000 tones/year and 42 crude ethanol plants of 2000 tones/year, along with some by-product utilization processes. The benefits of the system were analyzed. In the optimal system with a pure ethanol factory capacity of 50000 tones/year approximately 277 million Yuan profit could be obtained, employing 26,000 rural workers and saving 150,000 tons of CO2 emissions. It was also identified that clear economic and environmental benefits would result from utilization of the byproducts.

María et al (2009) [14] assessed agro industrial wastes for their suitability as fungus immobilization carrier for solid-state fermentation (SSF). The wastes included creosote bush leaves (Larrea tridentata), variegated Caribbean agave (Agave lechuguilla), lemon peel (Citrus aurantifolia), orange peel (Citrus sinensis), apple

pomace (Malus domestica), pistachio shell (Pistacia vera), wheat bran (Triticum spp.), coconut husk (Cocos nucífera), pecan nutshell (Carya illinoinensis), and bean residues (Phaseolus vulgaris). All of them were physical–chemically and microbiologically characterized. Physical–chemical tests consisted in the determination of the critical humidity point and the water absorption index; while the microbiological tests were based on the evaluation of Aspergillus niger Aa-20 growth rate in such materials. The study pointed out that coconut husk, apple pomace, lemon and orange peels were the materials of greater potential for use as immobilization carrier in SSF, since they have high water absorption capacity, and allowed good microorganism growth rate. Based on physical–chemical and microbiological tests it could be concluded that among the 10 agro industrial wastes evaluated, 4 of them, namely the apple pomace, lemon peel, orange peel, and coconut husk have great potential to be successfully used as immobilization carrier in SSF, for the production of industrially relevant metabolites. Such use would be an interesting alternative to add value to these residues besides to be of great economical advantage and an environmental–friendly way for waste management. Considerations must only be done regarding the initial glucose concentration, which if higher than 25 g/l affects the microorganism growth rate in lemon and orange peels, whereas apple pomace values higher than 50 g/l affect the microorganism performance. These facts should be taken into account when formulating a fermentation medium from these substrates.

7. Fermentation of municipalsolid wastes

Wen-Shiang et al (2012) [40] investigated the bioethanol production from sweet potato, the saccharification and fermentation conditions of co-immobilization of saccharolytic molds (Aspergillus oryzae and Monascus purpureus) with Saccharomyces cerevisiae. The immobilized yeast cells showed that at 10% glucose YPD (yeast extract peptone dextrose) the maximum fermentation rate was 80.23%. Viability of yeasts cells were 95.70% at a final ethanol concentration of 6%. Immobilization enhanced the ethanol tolerance of yeast cells. In co-immobilization of S. cerevisiae with A. oryzae or M. purpureus, the optimal hardening time of gel beads was between 15 and 60 min. Bioethanol production was 3.05-3.17% (v v-1) and the YE/s (yield of ethanol production/starch consumption) was 0.31-0.37 at pH 4, 30°C and 150 rpm during 13 days fermentation period. Co-immobilization of S. cerevisiae with a mixed cultures of A. oryzae and M. purpureus at a ratio of 2:1, the bioethanol production was 3.84% (v v-1), and the YE/s was 0.39 for 11 days incubation. However a ratio of A. oryzae and M. purpureus at 1:2 resulted a bioethanol production rate of 4.08% (v v-1), and a YE/s of 0.41 after 9 days of fermentation.

Thermotolerant ethanol-fermenting yeast, Saccharomyces cerevisiae KNU5377, isolated from sludge of a local industrial complex stream in Korea, was evaluated for its capability for lignocellulosic ethanol production from waste newsprint in high temperature (Park et al, 2010) [23]. In this fermentation, most of dry-defibrated waste newspaper was first saccharified at 50 °C for 108 h using a commercial cellulase and, then with the last addition of dry-defibrated newsprints to the pre-saccharified broth, simultaneous saccharification and fermentation (SSF) of 1.0 L of reaction mixture was carried out at 40°C, slowly being dropped from 50 °C, for further 72 h in a 5 L fermentor by inoculating the overnight culture of KNU5377. The maximum production of 8.4% (v/v) ethanol was obtained when 250 g (w/v)/L of dry-defibrated waste newspaper was used for ethanol production by SSF. These results suggest that S. cerevisiae KNU5377 is very useful for cellulose ethanol production by the SSF system.

Velásquez-Arredondo et al (2010) [37] produced ethanol from the hydrolysis of starch, cellulosic and hemi cellulosic material present in the banana fruit or its residual biomass. Four different production routes were analyzed: acid hydrolysis of amylaceous material (banana pulp and banana fruit) and enzymatic hydrolysis of lignocellulosic material (flower stalk and banana skin). The analysis considered banana plant cultivation, feed stock transport, hydrolysis, fermentation, distillation, dehydration, residue treatment and utility plant. The best indexes were obtained for amylaceous material for which mass performance varied from 346.5L/t to 388.7L/t; Net Energy Value (NEV) ranged from 9.86MJ/L to 9.94MJ/L and the energy ratio was 1.9MJ/MJ. For lignocellulosic materials, the figures were less favorable; mass performance varied from 86.1 to 123.5L/t, NEV from 5.24 to 8.79MJ/L and energy ratio from 1.3 to 1.6MJ/MJ. The analysis however showed that both processes can be considered energetically feasible.

Linde et al (2008) [12] obtained slurries from process streams in a starch-to-ethanol plant, Agroetanol AB in Norrkoing, Sweden. It was used to assess the potential increase in bioethanol yield if heat treatment followed by enzymatic hydrolysis

were applied to the residual starch-free cellulose and hemicellulose fractions. The effects of different pretreatment conditions on flour (the raw material), the stream after saccharification of starch, before fermentation, and after fermentation were studied. The conditions resulting in the highest concentration of glucose and xylose in all streams were heat treatment at 130°C for 40 min with 1% H2SO4. Mass-balance calculations over the fermentation showed that approximately 64%, 54%, 75% and 67% of the glucan, xylan, galactan and arabinan, respectively, in the flour remained water insoluble in the process stream after fermentation without any additional treatment. Utilizing only the starch in the flour would theoretically yield 425 L ethanol per ton flour. By applying heat pretreatment to the water-insoluble material prior to enzymatic hydrolysis, the ethanol yield could be increased by 59 L per ton flour, i.e. a 14% increase compared with starch-only utilization, assuming fermentation of the additional pentose and hexose sugars liberated.

Yue-Qin et al (2008) [43] produced ethanol from kitchen waste in this study. The process consists of freshness preservation of the waste, saccharification of the sugars in the waste, continuous ethanol fermentation of the saccharified liquid and anaerobic treatment of the saccharification residue and the stillage. Spraying lactic acid bacteria (LCB) on the kitchen waste kept the waste fresh for over 1 week. High glucose recovery (85.5%) from LCB-sprayed waste was achieved after saccharification using Nagase N-40 glucoamylase. The resulting saccharified liquid was used directly for ethanol fermentation, without the addition of any nutrients. High ethanol productivity (24.0 g l-1 h-1) was obtained when the flocculating yeast strain KF-7 was used in a continuous ethanol fermentation process at a dilution rate of 0.8h-1. The saccharification residue was mixed with stillage and treated in a thermophilic anaerobic continuous stirred tank reactor (CSTR); a VTS loading rate of 6 g l-1 d-1 with 72% VTS digestion efficiency was achieved. Using this process, 30.9 g ethanol, and 65.2 l biogas with 50% methane, was produced from 1 kg of kitchen waste containing 118.0 g total sugar. Thus, energy in kitchen waste can be converted to ethanol and methane, which can then be used as fuels, while simultaneously treating kitchen waste.

8. Conclusion:

In recent years it has been investigated that, instead of traditional feed stocks (starch crops), cellulosic biomass, including forest and industrial residues, agriculture waste and municipal waste, could be used as an ideally inexpensive and sufficient amount of sugar for production of ethanol by fermentation. Ethanol is comes into traditional fuel for transportation in last decades. Ethanol is less polluting and clean burning fuel. This review highlights the potentiality of industrial and forest waste, agriculture waste and municipal waste with different pretreatment methods. European Environment Agency assessed the potential of biomass for producing energy could increase into huge amount. Researchers mainly concern about cheapest and unused available resources to produce ethanol. Genetic engineering also works to improve the efficiency of microorganisms for increase yield as well as minimum cost of production. Commonly Pichia stipites and saccharomyces cerevisiae is used by different researchers. Enzymatic hydrolysis may be the most potent alternative process for saccharification of complex polymer contains feed. Forest, industrial wastes typically unused and available in large amount. Agricultural waste is renewable, less costly and available in nature. Agriculture waste do not demand separate land, water and energy requirement. They do not have food value as well. Most of the municipal solid waste contains starch, lignocellulos, hemicellulose and sugar that are sufficient for fermentation.

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