

Different Ways of Bioethanol Production; A Renewable and Alternative Energy Source (A Review)

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ABSTRACT

Gradual decrease in energy sources due to their increased consumption as a result of increase in world population and high environmental pollution has led to the invention of alternative renewable energy sources. Several studies are being carried out for this purpose. Different types of biofuels have been generated and among them, bioethanol is the major alternative source of energy. It posseses many special characteristics which make it unique from rest of the energy producing sources. It also has the ability to prevent the emission of certain dangerous gasses upon its burning, which are the main cause of environmental pollution such as global warming. Different types of feedstocks such as cereals, crops (sugar crops), lignocellulosic wastes and others including yeasts and algal biomasses have been studied for bioethanol production. This review gathers many different sources and methods for the production of bioethanol, techniques to enhance the production and ways to make it useful for the mankind.

Keywords: Bioethanol, Feedstock, Fermentation, Saccharification, Lignocelluloses

I. INTRODUCTION

Energy demands are increasing day by day due to increasing world population and industrialization. As the consequences, energy resources are becoming rare and so their cost is increasing from time to time. The only major source of energy are the fossil fuels [1]. The burning of these fossil fuels takes place in the form of coal, oil and natural gass. Carbon dioxide is emitted as the result of combustion of fossil fuels which is a type of green house gass and is becoming the major cause of global warming, increasing temperature on the earth. Awareness of the global climate change and the inevitable depleteion of energy supply has led to an increasing interest for the development of some renewable energy sources with lower environmental impacts [2]. Bioethanol in this aspect is considered as an important and attractive renewable and sustainable energy source which can perform much better than fossil fuel-derived fuels. Bioethanol possesses useful properties like fuel energy, evaporation enthalpy, high octane number (108) and wider range of flammability. Owing to these characteristics, bioethanol as a fuel,

provides greater effeciency than that of other fuels such as gasoline [3].

Bioethanol contains 35% oxygen, which causes the complete combustion of it and thus prevents the emission of carbon dioxide, nitrogen oxide and many other harmful green house gasses thus reducing the environmental pollution. Moreover, bioethanol causes much less toxicity to humans as compared to gasoline. It produces very low level of smog-producing compounds as a result of its combustion, thus reducing smog formation. It makes no or very less interference with ozone as a result of its low photochemical reactivity [4]. Bioethanol can be produced from a variety of feedstocks (fig. 1). In the past decade, researches and investigations have been done for bioethanol production. One of them done in USA and Brazil were proved very successful. In Brazil, bioethanol is produced by the first generation process which is cost-effective and gives high production yeild as the starting material i.e. sugar cane (already rich in sugars), doesn't need hydrolysis and fermentation needs no any defined closed system [5].

The worldwide production of bioethanol was started during the 2nd half of the last century probably in 1970, when a decrease in oil availability was noticed. In 1975, its production was less than billion liters but in 2006, it reached above 39 billion liters and it is believed to increase much more in the coming years [6]. Takagi et al. (1977) studied new fermentation processes for bioethanol production [7]. In 1974, Gulf oil company also introduced different methods for production of bioethanol [8]. The Zimbabwean Triangel Ethanol Plant initiated bioethanol production in 1980. It used to produce bioethanol, daily upto 120000 liters and 40 million liters annually. Due to local cultivation, sugar cane molasses was used as the major feedstock. After 12 years of working, this plant was shut down due to drought and unavailability of the feedstock. In 1982, Dwangwa Estate Plant in Malawi started producing bioethanol from sugar cane molasses as the main feedstock. It produed 15 to 20 million liters of bioethanol in a year. In Kenya during 1980's, the Muhoroni Plant also produced daily 45000 liters of bioethanol using sugar cane molasses but then it had to close due to some economical reasons [5,9].

Several substrates such as starch, lignocelluloses and different wastes can be used for bioethanol production [10]. At industrial scale, ethanol is produced by the hydration of ethylene, catalysed by some acids [11]. Industrial bioethanol production can also be carried out by two types of primary feedstocks which are (i) starch from cereal crops and juices, and (ii) molasses from sugar crops [12]. Besides, in the present years, bioethanol is also bieng produced by the use of algae [13], marine yeast [14], modified yeast strains and many other sources[15].

Bioethanol production by using crops as feedstocks compete with the food requirements. In Zambia, 95% of the cultivated maize is used as food. It is also the major food source in USA, and so it will cause an increase in cost and very little availability if it would be used as source for bioethanol production. Sugar cane and bagasse are used mainly for heat and power generation in Marituis. In this way if sugar is used as feedstock for bioethanol production purposes, then not only the power supply will be threatened but the food supply will also be decreased drastically [16]. Land availability is another challenge towards bioethanol production. The land is the major financial source for farmers and is the source of crop cultivation to be used as food. If this land is utilized, for example, bioethanol crop production, then both the food crises will originate and the farmers will lose their only source of living. Besides, we have to



Figure 1: Different kinds of feedstock used for bioethanol production

face several pollution problems due to loss of biodiversity, severe deforestation and organic soil erosion in order to make the land available for bioethanol crop production [9]. This review is an overview of the different methods used for bioethanol production. These methods have been developed to fulfill the fuel requirements and making them more economical for use by humans.

II. BIOETHANOL

Bioethanol is also called as ethyl alcohol or simply ethanol. Its chemical formula is C₂H₅OH. The processing of biomass through biochemical process i.e. hydrolysis and microbiological fermentation converts it into bioethanol. The biomass can be edible (cereal grains, sugar crops) and non edible (algae, marine yeast and lignocellulosic residue) feedstocks. These feedstocks have been classified as first, second and third generation feedstocks depending upon the carbohydrate containing source materials. Starch riched sources such as cereal grains and sugar crops (sugar cane, sugar beet) belong to the first generation feedstocks. Second generation feedstocks are non edible, lignocellulosic materials such as wheat straw, rice husk, switch grass, wood and many other similar substances [17]. In third generation feedstocks, come macroalagal biomass which include seaweeds [13]. Bioethanol is produced from these feedstocks through hydrolysis and the microbial fermentation processes. This bioethanol can be used as biofuel as well as in alcoholic beverages [18].

III. PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

Bioethanol production from non-edible lignocellulosic material such as wheat straw, rice straw, bagass, corn stover, wood, peels of fruits and vegetables have now become an emerging interest for researchers, as they do not compete with food materials and supplies. Lignocellulose is an organic material and is the principal structural part of all plants. It is mainly composed of cellulose, hemicellulose and lignin. (i)Cellulose is the main component of plant materials and an abundant organic molecule. It is also called as β -1-4-glucan and is a polymer of glucose. It is resistant to biological and chemical treatments. (ii)Hemicellulose is a polymer of pentoses, hexoses and sugar acids. In soft woods, its main sugar component is mannose and in hardwoods, xylose is the main sugar component. It is 25% to 35% of the total lignocellulosic biomass. (iii)Lignin which is the third major constituent of lignocellulosic material, is linked to both cellulose and hemicellulose. It works as glue and strengthens the lignocellulosic biomass structurally. It consists of three types of aromatic alcohols which are coniferyl, P-coumaryl and sinapyl alcohol [10, 19].

A number of different industries produce huge amounts of lignocellulosic wastes, which are agriculture and food, forestry, paper and pulp alongwith wastes from municipal solid wastes (MSW), and animal wastes [20]. Very large efforts are to be made for the conversion of these lignocellulosic residues to important products like bioethanol and many others [21]. The lignocellulosic biomass has to be gone through some processing for final production of bioethanol (Fig. 2). This include the pre-treatment methods for enhancing the yeild of fermentable sugars and to increase cellular reactivity. The pre-treatment methods also minimize the formation of inhibitory factors and help in the conversion of lignin into valuable co-products [22]. There are three types of pre-treatment methods i.e. physical, chemical and biological pre-treatment methods.

Physical pre-treatment methods involve the degradation of lignocelluloses by chipping, grinding and miling [23]. In addition pyrolysis [24] and ultrasounds are also very effective for the breakdown of lignocellulosic biomass [25]. In chemical pre-treatment methods, we use alkalies(bases), acids (both concentrated and diluted) [26], wet oxidation [27] and green solvents (ionic liquids, acidified organic solvents) to lyse lignocellulosic wastes and yeild fermentable sugars in a large quantity [28]. The third major type includes biological pre-treatment methods which use microorganisms and their enzymes to delignify lignocellulosic residues. Some of the microorganisms include mainly basidiomycetes such as *Daedalea flavida*, *Phlebia floridensis*, and *P.radiata* as well as white-, brown- and soft rot fungi [29].

The heart in ethanol production is fermentation during which the fermentable sugars (e.g. hexoses i.e. glucose, lactose, mannose and pentoses i.e. xylose, arabinose), obtained after pre-treatment of lignocellulosic residues, are converted into bioethanol [30]. Two types of fermentation methods are (i)separate hydrolysis and fermentation (SHF) and (ii)simultaneous saccharification and fermentation (SSF).In SHF and hydrolysis, a separate process is needed for the hydrolysed products to be fermented into bio-ethanol. It has the advantage that both the processes i.e. hydrolysis and fermentation can be monitored individually and optimum temperature for hydrolysis is 45-50°C and for fermentation it is 30°C. However, enzyme inhibiting end-products are formed during hydrolytic step and high cost β -glucosidase has to be added to overcome the problem, which makes this method unfit for use [31].





The simultaneous Saccharification and fermentation process combines both the saccharification of polymeric cellulose using enzymes to simple forms (glucose), and fermentation of these monomeric sugars by yeast to bioethanol in the same container. The advantages of this method are faster conversion of sugars into bioethanol, higher yields, and greater and improved bio-ethanal concentrations. Moreover, the costly addition of β -galucosidase are also not needed as the end products are

converted directly into bioethanol. The chances of contamination of the fermentation broth are very little due to presence of bioethanol in it. The major drawback of the procedure is that the conditions like pH and temperature has to be compromised [8]. Another process named consolidated bio-processing (CBP) combines all the bioconversion steps into one single step in the same vessel by using one or more than one microorganisms. This is a very cost effective process for bioethanol production [32].

IV. MARINE YEAST PROCESSING

According to recent studies, several different halotolerant biomass sources such as seaweed and sealettuce can be an excellent alternate for bioethanol production (Table 1) as they do not compete with edible crop materials. Recently 10 marine yaest strains are isolated from mangrove sediments on south-east cost of India. These include *Candida albicans, Candida tropicals, Debaromyces hansenii, Geotrichum sp., Pichia capsulata, Pichia fermentans, Pichia salicaria, Rhodotorula minuta, Cryptococcus dimennae* and *Yarrowia lipolyca.* Among these, *P. salicaria* is considered best for ethanol production. In one finding, bioethanol was produced by *P.salicaria* when 2% sawdust filtrates were hydrolyzed (by diluted phosphoric acid) after 120 hours of incubation. *Candida albicans* is considered the 2nd highest ethanol producing fungi [33]. Obara *et al.* (2012) reported marine yeast *S.cerevisiae* (C-19) a better chioce for the production of ethanol due to its high osmotic tolerance. This strain (C-19) of *S.cerevisae* was compared with other of its two control strains i.e. *S.cerevisae* NRBC 10217 and *S.cerevisae* K-7 in terms of fermentation for bioethanol production [34].

The finding revealed that *S.cerevisae* (C-19) was much better at producing ethanol than the two control strains. Another marine yeast strain related to *Candida sp.* was isolated from veraval, present on the west coast of India. This isolated strain grew well in the presence of 2-13% salt and was able to produce bioethanol from sugar cane bagass hydrolysate, galactose, and hydrolysate of a seaweed *Kappaphycus alvarezii* in the presence of high salt concentrations (2.5-15% w/v) and under a wide pH range (2.0-11.0). The fermentation process by using these isolated marine yeasts is cost effective as the additional energy consuming steps and desalting are not required when marine biomass is used for the production of bioethanol [35].

Yeast	Isolation source	Substrate	Hydrolysis method	Fermentation condition (sugar conc., temp., incubation time)	Ethan ol conc. g/L	Refe- rences
Candida sp.	Veraval, the West coast of India	Seaweed Sugarcane Bagasse Galactose	2.5% H ₂ SO ₄ , cooked at 100°C for 1h N/A	3.77% sugar, 30°C, 48 h 2.28% sugar, 30°C, 48 h 5% galactose, 30°C, 0-1% KCl, 24 h	17.6 21-24	[35]
S.cerevisiae	Mangrove soil, southeast coast of India	Sawdust	0.8% H ₃ PO ₄	6.84 mg/l sawdust, 30°C, 89 h		[36]
S.cerevisiae	Tokyo Bay, Japan	Paper shredder scrap	$\begin{array}{cccc} 3\% & H_2SO_4 & at & 121^\circ C \\ for & 1h & Enzymatic \\ saccharification \\ (cellulase for 2 days at \\ 50^\circ C and 150 \end{array}$	29.7% glucose from paper shredder scrap, 30°C, 72 h	122.5	[34]

Table 1: Marine yeast utilization for bioethanol production

		Sawdust	r.p.m.) NaOH 4% at 121°C for 30 min.	Immobilized 2% sawdust, 28°C, 120 r.p.m., 72 h	13 7.6
Candida albicans, C. tropicals, D. hansenii, Geotrichum sp.	Sediments, southeast coast of India	Glucose	N/A	28°C, 120 r.p.m., 96 h. Nonimmobilized	9.8- 28.5 [33]
Pichia capsulata, P. fermentans, P. salicaria,				28°C, 120 r.p.m., 96 h. Immobilized	13-47.3
R.minuta, C. dimennae and Y. lipolytica		Sawdust	NaOH 4% at 121 °C for 30 min.	2% sawdust, 28°C, 120 r.p.m., 72 h.	1.7- [37] 12.3

V. USE OF MODIFIED YEAST STRAINS

Improvements in bioethanol production have also been done through genetic modification of yeast strains used for this purpose. The major focus of genetic modification of yeast is on producing ethanol tolerant microorganisms for enhanced bioethanol production.Several researches have been adopted for producing new modified yeasts, from natural resources, which are resistant to different stressors such as high temperature, high acetic acid concentration, freezethawing and high salt concentrations, for production of bioethanol tolerant strains [38]. Different strategies have been proved helpful like genome shuffling (carried out by sporulation and hybridization), random mutagenesis (genetic material is modified by the use of a potent mutagenic agent) and exposure to the ultra violet radiations [39, 40, 41]. A technology gTME (global transcription machinery engineering) uses S.cerevisae to produce modified ethanol-resistant strains [42]. Moreover these strains have also been created by deletion mutant library (one gene knocked out) screening, and transposons mediated mutant collections have also been used for producing these modified yeast strains [43, 44].

Good results have been obtained regarding ethanol production by utilizing the modified strains rather than that of wild type. In the typical industrial ethanol processing, glycerol is the major by-product for which, yeast consumes an extra 4% sugars. This can be avoided by the deletion of GPD2 gene and over-expression of GLT1 gene in *S.cerevisae*. After this mutation glucose formation is reduced strikingly and ethanol production can be improved greatly. This improvement can also be achieved by substituting NAD+ dependent GPD1 gene by non-phosphorylating NADP+ GPD from bacillus cereus in the yeast, producing ethanol at industrial scale. As a result, glycerol levels are reduced and higher ethanol yeild is obtained [45].

VI. ALGAL SOURCES FOR BIOETHANOL PRODUCTION

Several researchers and entrepreneurs have reported the production of bioethanol by using some of the alternative feedstocks such as algal biomass (Table 2) instead of conventional crops such as corn and soybean. The main advantage of using algal biomass for bioethanol production is that it does not compete with the food materials. Microalgae are photosynthetic microorganisms which grow by using carbon dioxide, light and some nutrients (phosphorus, nitrogen, potassium) and produce a tremendous amount of carbohydrates and lipids which can then be utilized for different types of biofuels including bioethanol and many other important co-products [46]. Different types of algae have been reported for bioethanol production for example, Prymnesium parvum, Cholorococcum sp., Galidium amansii, Laminaria sp., Spirogyra, sp., Gracilaria, sp. and Sargassum sp. They don't need special conditions for growth but light, CO₂ and

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nutrients, and produce considerable levels of polysaccharides including starch and celluose. These are then hydrolyzed into sugar juices which are further fermented for bioethanol production [47].

During pre-treatment process, algal biomass produces different monosaccharaides by using certain enzymes. The most widely used method is acid pre-treatment. It is a promising method for bioethanol production and consumes very little amounts of energy. A high yield of sugars can be obtained by 7% (w/w) sulphuric acid pretreatment of Nizimudinia zanardini, a brown macroalgae, without any inhibitors formation [48, 49]. A lesser concentration of sulphuric acid (i.e. 5%) can produce bioethanol from pre-treatment of red seaweed Eucheuma cottonii yielding high amounts of sugars [50]. Besides, gamma radiation and enzymatic digestion can also be carried out for more improved pre-treatment processes [51]. The pre-treated algal biomasses are saccharified, suitably by the use of enzymes. Direct saccharification of biomass can also be carried out without pre-treatment process. It shows that pretreatment is not necessary in every case. Some of the enzymes that are used for saccharification of the biomass include amylases, cellulases, gluco-amylases

and viscozymes. However the biomass containing cellulose (such as green algae, are better saccharified by cellulases than any other enzymes. The disadvantage is that, it is expensive than other enzymes and high doses are required for an effective saccharification of celluloses [52].

A large number of different bacteria, yeast and fungi have been used for both aerobic and anaerobic fermentation after pre-treatment and saccharification of algal biomass. Anaerobic fermentation is best carried out by Z.mobilis and S.cerevisiae for bioethanol production. However some algae contain polymer which can be fermented in aerobic conditions only (e.g. mannitol) for which Zymobactor palmae is the microorganism of choice. Moreover, the agar present in certain red algae which is a polymer of galactose and galacto-pyranose, can also be the good sugar source including cellulose, alginate, mannitol, fucoidan and laminarian. Their fermentation produces a better yield of ethanol. Glucose and galactose extracted from red algae are also considered suitable for ethanol production [53, 54].

Table 2: Bioethanol production from algal biomass

Algae	Conditions	Bioethanol	References
Chlorococcum infusionum	Alkaline pre-treatment, temp. 120°C, S.cerevisiae	260g/kg algae	[56]
Spirogyra	Alkaline pre-treatment, synthetic media growth, saccharification of biomass by <i>Aspergillus niger</i> , fermentation by <i>S.cerevisiae</i>	80g/kg algae	[57]
Chlorococcum humicola	Acid pre-treatment, temp. 160°C, S.cerevisiae	520g/kg microalgae	[48]

Advantages of algal ethanol production

a) Greater CO₂ tolerance

- b) Less water is needed for algal cultivation
- c) No additional nutrients are required, rather, waste water containing nitrogen and phosphorus are enough for the cultivation of algae
- *d)* Able to grow even in harsh conditions like brackish water, saline and coastal sea water
- e) No pesticides or herbicides are required in algal cultivation [47]

Disadvantages

- a) A high energy input is required for harvesting algae
- b) Cultivation of algae is more expensive than that of other conventional crops
- c) A number of different techniques are required for cultivation and harvesting algal biomass like filtration, flocculation, centrifugation, floatation and sedimentation [55]

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VII. PRODUCTION FROM POTENTIAL JUICES

Several potential crops such as sugarcane, sugar beet, sweet sorghum and some fruits yields free sugar containing juices used as feedstocks in ethanol production. These sugar containing juices contain free sugars particularly sucrose, glucose, and fructose making them a cheaper source for bioethanol production in industry as compared to starchy grains or lignocellulosic materials [58]. Before the fermentation process with microorganisms, such as yeast, juice is extracted from sugar crops nourished with ammonium sulphate and nitrogen sources, sterilized and with pH and sugar concentration being adjusted. The yeast contains invertase enzyme in their periplasmic spaces, which breaks down sucrose, the main sugar component in fermentable juices, into glucose and sucrose during fermentation [59]. Sugarcane, a C4 plant possesses greater capability to convert solar radiation into biomass [60]. Its juice or molasses can be used as important feedstock for fuel ethanol production. Sugarcane juice containing organic nutrients, minerals and free sugars is an ideal feedstock for the production of bio-ethanol. This is the major source for bioethanol production in Brazil [12], while in India, molasses is the main feedstock for this purpose [61].

Sweet sorghum, also a C4 plant, is a potential energy crop, because of its high carbon assimilation (50g/m²/day). Its photosynthetic efficiency (activity) is very high owing to which, it can be cultivated in almost all areas having different climates. The production of sweet sorghum needs lower fertilizer and a little nitrogen, has short growing period (4-5 months), and is capable to tolerate both drought and cold temperatures. Moreover, both of its grains and juices can be used directly for ethanol production and do not require the pre-fermentation processing. Based on these advantages, sweet sorghum is considered a promising and one of the most beneficial crops for the production of bioethanol [62]. Sugar beet and beet molasses also contain free sugars which can be used for bioethanol production through fermentation and they do not need to be modified. Besides this, many different by-products and intermediates of sugar beets i.e. beet pulp, molasses, raw juices, and thick juices have a great potential for bioethanol production [63, 64].

Sugar fermentation is that important part of bioethanol production which is totally dependent on the microorganisms. So these microorganisms are the potential bio-agents in fermentation technology (Table 3). The criteria for these microorganisms to be chosen is the higher ethanol yield, enhanced ethanol productivity, a fine and increased growth in simple and inexpensive media, tolerance to ethanol, inhibition of contaminant growth and ability to grow in undiluted fermentation broth. and resistance to inhibitors [65].The microorganisms used for ethanol production from sugar juices, include Saccharomyces diastecticus, S. cerevisae, Pichia kudriavzevii, Zymomonas mobilis, Escherichia coli strain KO11, Klebsiella oxytoca strain P2 and Kluyveromyces marxianus. Among these, S.cerevisae is the microorganism of choice, as it fulfils most of the criteria for ethanal productions described above.

Three types of fermentations have been proposed which produce most of the bioethanol, are (i)Batch, (ii)fed Batch. and (iii)continuous fermentation. These fermentation methods are selected on the basis of the microorganisms used and the nature of the feedstock. The simplest fermentation process is batch fermentation. It is cost effective, easily sterilized, and requires less control measures. Fed batch fermentation mode posseses characteristics of both batch and continuous processes and hence is employed at industrial level for bioethanol production [66]. Continuous frementation is also a cost effective procedure and is better than batch fermentation process [67]. During the fermentation of sugars for bioethanol production, free cells of suitable microorganisms are used. But now, the improvements have been done by using immobilized cells instead of free cells. The use of immobilized cells provides better ethanol yield with minimum inhibitory effects produced by high concentrations of substrates and products [68].

Table 3: Processing of potential juices for bioethanol production

	Feedstock	Initial sugar (g/L)	Fermentation mode	Microorganisms	Conditions	Ethanol Concentrati on (g/L)	References
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	200	Batch	K.marxianus DMKU 3-1042	40°C for 42- 96 h. pH=5.0	67.9	[60]
	166	Batch	P.kudriavzevii	40°C for 24h. pH=5.5	71.9	[69]
	173	Repeated batch	S.cerevisiae	30°C for 32 h.	89.73	[60]
Sugarcane Juice	220	Batch	<i>K.marxianus</i> DMKU 3-1042 and <i>S.cerevisiae</i> M30	37°C-40°C for 72 h. pH=5.0	77.3-89.4	[60]
	180	Continuous	Strains of Saccharomyces sp.	30°C for 117 h. pH= 5.0	13.3-19.4	[70]
	200	Continuous	S.cerevisiae IR-2	30°C for 72 h.	90	[70]
Sweet	190	Fed-batch	S.cerevisiae TSTR 5048	30°C for 108 h.	116.62- 120.28	[66]
juice	240-320	Batch	S.cerevisiae NP01	30°C for 40- 72 h. pH=4.9	120.68	[71]
Watermelon juice	183-409	Batch	Dried yeast	32°C for 36- 160 h. pH 3.1-5.0	83.2	[72]
Sugar beet juice	200	Batch	S.cerevisiae, Candida brassicae and Z.mobilis	30°C, pH=6.5		[63]

VIII. CONCLUSIONS

Bioethanol production from all these sources is a great step towards the evaluation of renewable and alternative sources of energy in order to fulfill the energy demands. However, the production of bioethanol from food materials such as sugar crops is a challanging situation as this comes in competition with the food requirements. For this purpose, researches are being made to use sources other than the edible materials such as lignocellulosic wastes, industrial wastes, and water sources like algae and marine yeasts. A comprehensive process analysis is needed to enhance ethanol production at industrial scale, increase its stability and make it safe for use in order to cope with the energy crisis in the world.

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