

## Critical review on ethanol producing feedstock and methods

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**Abstract.** Dwindling of fossil fuels and the change in global climate have led humankind to explore alternative energy resources. In the force to explore inexhaustible and sustainable resources, research in the domain of solar, wind, tidal, biomass and geothermal are delved into. The energy derived from these various domains proved to be the solution to the greater cause of climate change. Among all these various sources of energy, biomass have tremendous potential. Bioethanol produced from biomass is an attractive biofuel having great prospect for energy security and environmental safety over fossil fuels. The conversion process of biomass to ethanol have improved significantly over the years to make it a viable option in transportation sector thereby reducing dependency on petrol and diesel. The major steps in typical conversion process include pre-treatment, enzymatic hydrolysis, and fermentation of sugars, greatly influenced by microbial strains. Yeast *saccharomyces cerevisiae* and a bacterial species, *zymomonas mobilis*, are the two most widely used in ethanol fermentation technology. The final yield of ethanol is directly dependent on the various factors affecting the production process. This comprehensive review study gives an overview of second-generation bioethanol production in regard with the various factors affecting its overall production process.

### Introduction

The two significant challenges currently present in the world are (1) providing enough energy sources and (2) protecting the environment. In the past few decades, humankind's intense use of difficult-to-recover petroleum reserves for commercial and personal use has heightened environmental and energy security issues. The dependency on fossil fuels by the world economy is because it is the primary source of energy (about 80%) [1]. Uncontrolled use of non-renewable fuels harms nature because of the greenhouse emissions and environmental pollution resulting in global warming [2]. In 2021 globally coal accounted for over 40% of the overall growth in CO<sub>2</sub> emissions. The highest increase in CO<sub>2</sub> emissions in 2021 occurred in heat production and electricity sector since the utilization of fossil fuels shoot up to help meet the growth of electricity demand [3]. It is imperative to slow down fossil fuel dependency as it is a limited resource and will get exhausted soon. Thus, increasing demands of fuel and extensive climate change issues are the main driving factors that is shifting the world's attention towards finding alternative energy



resources. These alternative energy resources can result in reduced dependency on fossil fuels and thereby pave the way towards sustainability. Regarding this, The Sustainable Development Goals (SDGs) were adopted by United Nations in 2015 to end hunger, make energy affordable and clean, form sustainable cities and communities, and ensure that by 2030 people all around the globe enjoy peace and prosperity [4]. Also, governments are promoting the concept of circular economy, in which the primary motive is to ensure resource efficiency and less waste. It is a new way of producing value where resources are used more efficiently by using them multiple times instead of just once [5].

Alternative energy sources are prime solution to tackle the increasing prices of fossil fuel supplies. This is the reason production of biofuel is advocated as a resolution to escalating crude oil costs and viable choice to non-renewable resources. To enhance India's energy security, Government of India aim to reduce import dependency on fuel by extensively encouraging the Ethanol Blended Petrol (EBP) Programme. India has achieved the target of 10% ethanol blending in petrol in June 2022, five months ahead of the tentative schedule [6]. This policy further is amended in last year, namely National Policy on Biofuels-2018 Amendment, 2022 as per which oil companies shall sell Ethanol Blended Petrol (EBP) with ethanol percentage being 20% throughout the country from 1st April 2023 [7]. As per Pradhan Mantri Ji-Van Yojana, lignocellulosic surplus biomass availability in India is about 12-16 crore tons per annum which if utilized properly, there's possibility to yield 2500 to 3000 crore litres of ethanol annually [8]. This can further bring down India's dependency on imported crude oil significantly. In the effort to combat increasing fuel prices and pollution, flex fuels have been gaining footing as alternative and environment friendly fuels. Usage of ethanol as flex-fuel will curb the dependency on imported crude oil as well as carbon footprints. In 2022, Toyota launched first of its kind project on Flexi-Fuel Strong Hybrid Electric Vehicles (FFV-SHEV) which would function on 100% petrol as well as 20% to 100% blended ethanol and electric power [9].

Biofuels are generally divided into first generation, second generation, third generation and fourth generation. Bioethanol obtained from first-generation biofuel comes from starchy food crops, namely sugar cane, wheat, maize. Thus, its production disputes with the accessibility of food and cultivable land [10]. Bioethanol obtained from lignocelluloses are biofuels belonging to the second generation, namely sugarcane bagasse, corn husks, and rice husks. Third generation biofuels are obtained from various species of algae biomass. Algae has a high lipid content and requires very less space for production, which makes them a viable option for biofuel production. Also, the fourth-generation biofuels are algae which are modified genetically [11]. The availability of lignocellulosic is in large quantities and is also easily accessible which is why biofuels produced from these biomasses by means of the alcoholic fermentation of sugar is getting more attention [12,13]. Lignocellulosic biomass (LCM) mainly consists of cellulose (~50%), hemicelluloses (~30%) and lignin (~20%). These components together constitute about 90% of the dry matter in lignocelluloses, and the remaining percentage is composed of extract and ash. These energy sources have tremendous potential in the shape of biofuel, more specifically bioethanol [14]. However, the conversion of sugars, starch and lignocellulosic biomass into bioethanol is different, regarding obtaining sugar solutions. To get fermentable sugars, raw materials which are sugar based require an extraction process, whereas starchy crops must undergo hydrolysis for the conversion of starch into glucose. In the case of lignocellulosic biomass, pre-treatment before hydrolysis is conducted to alter the cellulose structures for smooth enzyme accessibility [15].

### **Production process of ethanol from lignocellulosic biomass**

Lignocellulosic are formed of three main components namely cellulose, hemicellulose and lignin. Except lignin, composition of cellulose and hemicellulose is of chains of sugar molecules. These chains when hydrolyzed leads to the production of monomeric sugars. Some part of monomeric sugars can be further fermented using baker's yeast. To make the production economically

successful, high ethanol yield is a prime requirement. Lignocellulosic biomasses are abundant and easily available and is a great alternative to petroleum and other natural resources. These are primarily comprised of non-food crops (namely switchgrass and miscanthus), agricultural wastes (namely rice husks, sugarcane bagasse, coconut husks), forest residues, energy crops. The chemical composition of lignocellulosic biomass consists of cellulose (30–60%), hemicellulose (20–40%) and lignin (10–25%), along with tiny portion of ash, protein, and extractives [16]. Biological transformation of lignocellulosic biomass to bioethanol has attracted more and more attention over the period of years. The procurement of bioethanol involves all aspects of the key processing steps viz., pre-treatment, enzymatic hydrolysis, and fermentation [17]. However, its conversion to second generation ethanol is very demanding in comparison to first generation ethanol essentially owing to impenetrable characteristics of biomass matrix, ineffective disintegration of carbohydrate-lignin structure composite, high lignin content. For the conversion of lignocellulosic biomass, most commercial second-generation production units involves downstream and upstream technologies. The pathways of production initiate with upstream pre-processing of biomass feedstocks (e.g., sieving, cutting, milling, size reduction, grinding); pre-treatment for the eradication of the legitimate roadblock and further making it attainable to enzymes. After this enzymatic saccharification is done which releases free monomeric sugars. In case of downstream technologies, the fermentation of monomeric sugars is conducted via microorganisms. The obtained fermented broth is then distilled and further dried to obtain complete ethanol for blending motives [18].

#### Pre-treatment process used for Bioethanol process

Pre-treatment step alters the macroscopic, sub-microscopic and microscopic structures of the biomass. This step is costly and critical. Pre-treatment process remove hemicellulose along with lignin from the lignocellulosic matrix. It further decreases the crystallinity of cellulose, at the same time increasing the surface area of biomass [19]. To facilitate the hydrolysis of cellulose for final production of ethanol, various pre-treatment techniques have been developed namely physical, physiochemical, and chemical pre-treatments. Physical treatment includes hydrolysis and comminution whereas chemical pre-treatment is conducted using alkali, acid, ozone, and solvent based procedures. Another type of pre-treatment namely physiochemical exists which is basically the amalgamation of chemical and physical methods such as ammonia fibre expansion and steam explosion. The main purpose of this procedure is to disarray the complex structures of lignocellulosic biomass and its major components into simple sugars, which will ensure fruitful bioconversion process. The various pre-treatment methods namely include steam explosion, acid pre-treatment, alkaline pre-treatment, thermal pre-treatment, ammonia fibre explosion (AFEX), wet oxidation pre-treatment, carbon-dioxide explosion pre-treatment, ozonolysis pre-treatment, organosolvent pre-treatment and aqueous ammonia solution pre-treatment. In recent times new pre-treatment methods have been developed to value lignocellulosic biomass. For the separation of lignocellulosic feedstock to produce pure cellulose and lignin portions in a feasible biorefinery, deep eutectic solvent (DES) is a prominent alternative proposition. This approach reveals the ionic characteristics which are tough and being ideal like ionic liquids for recovery and reuse [17].

#### Cellulose Hydrolysis

Hydrolysis process is conducted after pre-treatment to crack down the biomass into fermentable sugars. The two hydrolysis methods which are most used are acidic and enzymatic. Acid hydrolysis is categorized into dilute and concentrated hydrolysis. Dilute acid hydrolysis is conducted using lower acid concentration at elevated temperature. On the other hand, concentrated acid hydrolysis is done in high acid concentration at lower temperature. Enzymatic hydrolysis needs enzymes to hydrolyze the feedstocks into fermentable sugars. The three commonly used

types of enzymes are endo-β—1,4-glucanases, cello-biohydrolases and β-glucosidases. The cellulase enzyme activity is impacted by the source of enzyme and its concentration. The degradation of cellulose into reducing sugars will be at mild conditions of pH: 4.8-5.0, temperature: 45-50 °C. The enzymatic hydrolysis efficiency is greatly influenced by optimized conditions such as time, temperature, pH, substrate concentration and enzyme loading [20]. Enzymatic hydrolysis is a relatively slow procedure; however, it has the upper hand of no by-product being throughout the entire procedure. The use of cellobiase of *Trichoderma reesei* and cellulase has been reported to hydrolyze sugarcane after alkaline delignification [21]. However, to exhibit the fibers for enzyme accessibility pre-treatment is necessary before enzymatic hydrolysis.

### Fermentation

To produce alcohol, fermentation of hydrolysate from lignocellulosic biomass is prime necessity. Regarding this, pentose-utilizing negative yeast strains and pentose-utilizing yeast strains have been used. *Zymomonas mobilis*, *Escherichia coli*, *Kluyveromyces marxianus*, *Z. bailii*, *B. clausenii*, *Scheffersomyces stipitis* and *S. cerevisiae*, microbial strains have been build using recombinant DNA technology as they are competent of utilizing hexose and pentose sugars making the entire process efficient [22–24]. Fermentation of biomass is conducted mostly via separate hydrolysis and fermentation (SHF) and simultaneous hydrolysis and fermentation (SSF). In the separate hydrolysis and fermentation (SHF) process two steps are involved: hydrolysis and fermentation. In hydrolysis method, sugars are released from cellulose after pre-treatment. In fermentation, microbial strain used for conversion, converts released sugar into ethanol. Each and all steps are conducted separately at optimal conditions. In case of simultaneous hydrolysis and fermentation (SSF), hydrolysis and fermentation processes are combined and performed in one vessel simultaneously. The inhibition caused by the accumulation of sugars are eradicated in this process as the sugars released are transformed into bioethanol instantly with the help of appropriate microbial strains. The eradication of sugar accumulation results in increasing the extent of hydrolysis process and overall decrease the time taken in the step [25].

### Literature review on bioethanol production

This section discusses the process and quantity of bioethanol production utilizing feedstocks from various agricultural and industrial fields, refer Table 1. Table 1 also discusses the process of pretreatments at varying conditions while using enzymatic hydrolysis and fermentation process for maximizing the bioethanol yield.

*Table 1. Literature Review on Bioethanol Production*

Feedstocks	Descriptions	References
Wheat straw	Subcritical water pre-treatment is conducted (220.5 °C; 22.0 min). Further feedstock undergoes separate high solid (15%) hydrolysis and fermentation (SHF) is done. Bioethanol obtained 37.0 g/L	Chen et al. [26]
Bamboo	<i>Dendrocalamus sinicus</i> species of bamboo is subjected to sulfomethylation-aided phosphoric acid pre-treatment. Cellic Ctec2 and baker’s yeast is used for fermentation. Ethanol obtained is 13.26 g/L	Jin et al. [27]
Whole plant cassava	Hydrothermal pre-treatment is conducted (180 °C; 2 MPa; 60 min). Fermentation of C <sub>5</sub> integrated cellulosic sugar and starch in simultaneous saccharification and fermentation method (SSF).	Lyu et al. [28]

Polyploidy potato straw	Two pre-treatments (8 min liquid hot water; 5% CaO) at minimized conditions. Maximum bioethanol yield of 24 % (% dry matter)	Madadi et al. [29]
Wheat and rye stillages	Pre-treatment method is microwave-assisted with dilute acid. Produced >156 mg/g glucose, rated microwave power 300 W (15 min, 54 PSI in 24 h process), 48 h of fermentation with <i>S. cerevisiae</i> . Bioethanol obtained is 20 g/L.	Mikulski and Kłosowski [30]
Cotton stalk	Pre-treatment is done in hydrothermal and organosolv processes, thereafter pre-hydrolysis with use of 80 FPU/g cellulose (at 50 °C, at pH 5.0, for 6 h). After pre-treatment 15 mg yeast per gram undergoes fermentation at 30 °C with pH level of 5.0. Bioethanol obtained is 47.0 g/L	Dimos et al. [31]
Sugarcane Bagasse	Aqueous ammonia soaking pre-treatment is done (50 °C for 48 hr). Separate hydrolysis and fermentation: hydrolysed via fed batch at 40% high solids loading. Microorganism used is Cellic Ctec2. Glucose and xylose (96 hr hydrolysis) obtained is 157.65 g/l and 57.12 g/l respectively.	Raj and Krishnan [32]
Sugarcane Bagasse	Pre-treated with hydrodynamic cavitation, further assisted alkaline hydrogen at optimum conditions. Enzymes used for hydrolysis is cellulase. For fermentation <i>Scheffersomyces stipitis</i> NRRL-Y7124 is used. Bioethanol produced is 31.50 g/L.	Hilares et al. [33]
Eucalyptus biomass	At 150 °C, alkaline extrusion pre-treatment is conducted to achieve highest xylan and glucan conversion through enzymatic hydrolysis, about attaining 40% and 70% yields.	Duque et al. [34]
Sugarcane Bagasse	During pre-treatment process at 140 °C for 1 hr, 5% Na <sub>2</sub> CO <sub>3</sub> solution was used. In the hydrolysis step glucose obtained was 97.6%. At 37 °C for 72 h fermentation is done thereby bioethanol obtained is 7.27 g/L.	Nosratpour et al. [35]
Corn cob	<i>Spathaspora passalidarum</i> U1-58, is used to ferment the pre-treated corn cob. It uses pentoses and hexoses. Here, SSF gave higher yield value than SHF. After 96 hr of fermentation values obtained were 42.46 g/L and 53.24 g/L, respectively	Yu et al. [36]
Water Hyacinth	Pre-treatment is done with dilute H <sub>2</sub> SO <sub>4</sub> separate hydrolysis and fermentation. Fermentation is done by the combination of <i>S.cerevisiae</i> (MTCC 173) and <i>Z. mobilis</i> (MTCC 2428). The obtained ethanol was 13.6 mg/ml.	Das et al. [37]
Green coconut husk fibers	The pre-treatment method used is alkaline method, followed by enzymatic hydrolysis. In fermentation process <i>S. cerevisiae</i> were used. For fermentable sugars the bioethanol conversion efficiency was high as 59.6%.	Cabral et al. [38]

Water Hyacinth	NaOH pre-treatment is done, followed by separate hydrolysis and fermentation and simultaneous hydrolysis and fermentation. Strain used is control <i>S. cerevisiae</i> and <i>Kluyveromyces marxianu</i> K213. Maximum ethanol yield obtained is 7.34 g/L.	Yan et al. [39]
Rice straw	CO <sub>2</sub> gets incorporated in ammonia explosion pre-treatment at conditions: 14.3% ammonia, 2.2MPaCO <sub>2</sub> , 165.1°C for 69.8 min. The obtained glucose yield is 93.6%. Bioethanol obtained is 97% reportedly after simultaneous saccharification and fermentation method (SSF)	Cha et al. [40]

### Conclusion

Lignocellulosic wastes are a great source to produce bioethanol. However, the main challenge of researchers is to attain greater yield of bioethanol via economically achievable pre-treatment/hydrolysis processes. Pre-treatment process of the feedstocks is a very crucial footstep in bioethanol generation. These methods in most cases are costly and high energy consuming, which acts as a vital obstacle in the application of these propitious feedstocks on a commercially grand measure. To make it more effective, process integrations are conducted, however in practical scenario to make it more economically viable implementation of these advances in small scale or commercial scale would be more suitable regarding growing a sustainable lignocellulosic ethanol industry.

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