BIOETHANOL PRODUCTION FROM AGRICULTURAL WASTE

Abstract

The globe is looking for fuels other than petroleum-derived ones to lessen its reliance on non-renewable resources. Nowadays, ethanol made from maize grain (starch) and sugar cane (sucrose) is the most widely used renewable fuel. Since the availability of these raw materials is anticipated to reach a limit soon, lignocellulosic biomass is viewed as a promising feedstock for ethanol supplies in the future. But the creation of commercial processes that use biomass is hampered by both financial and technical issues. Innovations in technology are underway to enable the economical transformation of biomass into chemicals and fuels. Some of these technologies are robust and efficient fermentative microorganisms, extremely effective cellulases and hemicellulases and inexpensive thermochemical treatments. Commercialization now has greater promise due to the numerous advancements made in the last few years. By creating bioresearch energy and utilizing wastes to make bioethanol, the technique will assist combat the challenges posed by the depletion of fossil fuels and maintain a clean environment. excellent. environmentally An beneficial substitute for non-renewable fuels is bioethanol, which is made from garbage. Greenhouse gas emissions from using conventional gasoline are decreased when a percentage of bioethanol is added. Furthermore, current automobiles operate flawlessly on bioethanol blends without requiring any engine modifications, contributing to energy savings and a cleaner environment. Hence, bioethanol is a potential

alternative energy source in future as there is a strong need for sustainable energy sources to decrease reliance on foreign petroleum oil.

Keywords: Fossil fuels, bioethanol, lignocellulose, starch and sucrose

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I. INTRODUCTION

In India, about 350 million tonnes of agricultural waste is generated annually. According to estimations made by the Ministry of New and Renewable Energy, this waste can produce green fertilizer for use in agriculture (Goel *et al.*, 2022). It can generate more than 18,000 MW of power annually. Globally about 1.3 billion tonnes of food products for human consumption gets wasted or lost yearly. For example, total world potato waste is generated to be 12 million tonnes per annum, of which 2 million tonnes of potato waste is generated in India alone (Anon., 2020). This potato waste, in turn, generates obnoxious gases and greenhouse gases, besides foul odour, around the landfill sites.

Several technologies were to convert agricultural waste into wealth through the development of biocommodities with potential market demand, such as fermented beverages, single-cell proteins (SCP), single-cell oils (SCO), biocolours, flavors, polysaccharides, bioethanol, biogas, biopesticides, plant growth regulators, and biohydrogen through microbial processing (Panda *et al.*, 2017).

The world economy is highly dependent on various fossil energy sources, such as oil, coal, natural gas, etc., to produce fuel, electricity, and other goods (Uihlein and Schbek, 2009). (Ballesteros *et al.*, 2006). A drastic increase in the level of greenhouse gases in the earth's atmosphere has dramatically increased due to excessive usage of fossil fuels causing environmental damage. In this scenario, renewable sources might serve as an alternative, and petroleum-based fuels can be replaced by renewable biomass fuels such as bioethanol, biodiesel, bio-hydrogen, etc., derived from sugarcane, corn, algae, other agricultural wastes, fruits, and vegetable wastes, etc.

II. BIOETHANOL AND ITS IMPORTANCE

Bioethanol is a liquid biofuel produced by microbial fermentation of several different types of feedstocks, such as corn, soybeans, wheat straw, woodchips, fruits and vegetable wastes, and, more recently, microalgae. Bioethanol is non-toxic, biodegradable, and does not cause environmental pollution compared to fossil fuels. The conversion of waste to bioethanol can reduce the emission of greenhouse gases (Stichnothe and Azapagic, 2009). Waste materials containing cellulose, lignin, and lignocellulosic can produce bioethanol. In addition, biomass containing high carbon content, including waste, can be used to produce bioethanol by converting polysaccharides into simple fermentable sugars. The yeast fermentation of these sugars can result in the production of bioethanol. North America and Brazil produce large quantities of bioethanol as a transportation fuel. It is necessary to increase the production and use of bioethanol as an alternative to petroleum fuel.

There are many advantages of biofuel as a bioenergy source. Biofuel is considered carbon neutral due to the release of carbon dioxide while burning, which is equal to the amount that the plants absorb, and they do not contribute to the increase of global warming. Biofuel encourages farm income, reduces energy costs, and promotes further rural development while pleasing the environmental community. Production of biofuel replaces the usage of high price petroleum. The bioethanol production process is classified into three generations.

- First-generation bioethanol
- Second-generation bioethanol
- Third-generation bioethanol
- 1. First-Generation Bioethanol: First-generation ethanol was produced mainly from plant sugars or starches directly from food crops. Corn, wheat, and sugarcane were the primary feedstocks used. Sugar-based ethanol plants are predominantly produced in Brazil from sugarcane, and starch-based ethanol was produced generally from corn and grains significantly in the USA, followed by other ethanol-producing countries such as China, Canada, France, Germany, and Sweden (Arifin *et al.*, 2014).

The United States alone accounted for 58% of ethanol production, followed by Brazil (28%), China (3%), Canada (2%), and Thailand (1%); the European Union, led by France and Germany, accounted for 6 % of global production (Niphadkar *et al.*, 2017).

The main disadvantage of first-generation biofuel is the rising food prices due to an increase in the production of these fuels. Therefore, there is a search for more efficient and productive alternatives. Plant waste biomass, which mainly contains lignocellulosic materials, has the potential to produce novel biofuels known as second-generation biofuels.

- Drawbacks and Current Status: Corn is the foremost source of ethanol production, especially in the US, where 40% or more of the corn crop was used for such production. Corn is the staple food in many developing and developed countries, leading to a global increase in food prices and even hunger. The same problem also results when sugarcane is used as feedstock. Their cultivation requires the use of pesticides and fertilizers, which is costly and results in soil and water contamination. So environmental hazards posed another constraint in production. In addition, the production rate of ethanol with corn as feedstock is slow, and energy yield is also relatively low (20 % net yield).
- 2. Second-Generation Bioethanol: Second-generation bioethanol production used 'plant biomass' that was considerably cheaper, abundant, and did not present food-related conflicts (Gomez et al., 2008). Second-generation ethanol production processes were designed in such a way as to avoid food-versus-fuel conflicts and focused on agricultural residues and forest wastes mainly comprising different types of lignocellulosic (Lennartsson et al., 2014). The second-generation bioethanol production processes were immature initially, but with advancements in bioprocess strategies, cost reduction, and availability of sustainable resources, they developed into a profitable venture for a few producers. E.g.: Borregaard Company (Norway) is often considered the largest second-generation ethanol-producing unit (Rodsrud et al., 2012).
 - Drawbacks or Production Constraints of Second-Generation Bioethanol: The primary concern with second-generation bioethanol production was the sugar degradation and energy consumption in pre-treatment operations, making the overall process costly (Palacios-Bereche *et al.*, 2013; Dias *et al.*, 2014). The need for efficient microorganisms for simultaneous C₅ and C₆ fermentation into bioethanol is also a

problem. Again, the enzymes used for the saccharification process were costly, adding to the overall production cost.

- **3.** Third-Generation Bioethanol: Third-generation bioethanol uses high-carbon embedded biomass for production purposes. Seaweed and marine algae like *Enteromorpha* species which contain 70% carbohydrate on a dry weight basis, can be used for bioethanol production (Nahak et al., 2011) reported. Borines *et al.* (2013) carried out the degradation of polysaccharides from *Sargassum* spp. by optimizing pretreatment conditions in terms of glucose and reducing sugar, and producing ethanol (10–15%). Due to the high biomass conversion potential (46,760–140,290 L/ha), more research is being done on producing third-generation biofuels, especially from macro or microalgae (Chaudhary *et al.*, 2014).
 - Drawbacks, New Approaches, and Current Status: The chemical analysis of different macroalgae in the late 1990s revealed carbohydrate contents of 25–50 % in green algae, 30–60 % in red algae, and 30–50 % in brown algae (Sarkar *et al.*, 2012; Karimi *et al.*, 2013). In macroalgae species such as *Ascophyllum, Porphyra*, and *Palmaria*, the polysaccharide contents can be as high as 70–76 %. The major drawback of algal biorefining was that it did not directly yield fermentable sugars, and a further optimized pre-treatment was a prerequisite.

Bioethanol Generation	Biomass Source	Ethanol Yield (L/t)	Reference	
First Generation	Sugar beet Sugar cane Cassava Maize Rice Wheat	110 (L/t) 70–75 (L/t) 137–180 (L/t) 400 (L/t) 430 (L/t) 340 (L/t)	FAO, 2008	
Second Generation	Corn stover Wheat straw Sugarcane bagasse Juice from <i>Agave</i> <i>americana</i> leaves Rice straw	362–456 (L/t) 406 (L/t) 318–500 (L/t) 34 (L/t) 416 (L/t)	Corbin <i>et al.</i> , 2015	
Third Generation	Microalgae Brown seaweeds (macroalgae) Seagrass (macroalgae) Green seaweeds (macroalgae) Red seaweeds (macroalgae)	167–501 (L/t) 12–1128 (L/t) 747 (L/t) 72–608 (L/t) 12–595 (L/t)	Ramachandra and Hebbale, 2020	

Table 1: Approximate Ethanol Yields from Different Feedstock.

Among the three-generation process of bioethanol production, the second-generation process comprises a wide range of novel biofuels based on new feedstocks from lignocellulosic materials, which include agricultural wastes (e.g., straw), energy crops (e.g., Miscanthus, poplar), forestry products and wastes and parts of municipal solid waste. Hence second generation (2G) bioethanol production process is an attractive alternative for biowaste utilization (Niphadkar *et al.*, 2017)

III. RAW MATERIALS USED FOR BIOETHANOL PRODUCTION

Raw materials that can be utilized for bioethanol production can be classified based on their chemical composition, *i.e.*, carbohydrate sources can be classified into three groups:

- Sugar-containing raw materials: sugarcane, sweet sorghum, molasses, whey, sugar beet
- Starch-containing feedstocks: grains such as wheat, corn, tubers such as cassava
- Lignocellulosic biomass: agricultural waste like straw, crop and wood residues, etc. (Mussatto *et al.*, 2010; Sain, 2020).

However, first-generation feedstocks containing sugar and starch- products compete with their use as food or feed and will influence their supply. Therefore, lignocellulosic biomass (second generation) can be utilized as an alternative feedstock for bioethanol production since they are readily available, low in cost, and widely distributed, and will not compete with food and feed crops (Tomas-Pejo *et al.*, 2011).

IV. PRETREATMENT OF BIOMASS FOR BIOETHANOL PRODUCTION

The most critical processing challenge in biofuel production is the pretreatment of biomass. Lignocellulosic biomass has three main constituents: hemicellulose, lignin, and cellulose. Pretreatment methods include solubilization and separating one or more of these biomass components. It makes the remaining solid biomass more accessible for further chemical or biological treatment (Demirbas, 2005). The pretreatment is done to break the matrix to reduce the degree of crystallinity of the cellulose and to increase the fraction of amorphous cellulose, which is the most suitable form for enzymatic attack (Sanchez and Cardona, 2008).

The goals of an effective pretreatment process are production of sugars directly or subsequently by hydrolysis

- To avoid loss and degradation of sugars formed
- To limit the formation of inhibitory products
- To reduce energy demands
- To minimize costs

The four fundamental pretreatment techniques employed are physical, chemical, physicochemical, and biological treatments. Generally, a combination of these processes is used in the pretreatment step.

1. Physical Pretreatment

- **Mechanical Size Reduction:** The first step for ethanol production from agricultural solid wastes is comminution through milling, grinding, or chipping to reduce cellulose crystallinity (Sun and Cheng, 2002) and improve the efficiency of downstream processing. Wet milling, dry milling, vibratory ball milling, and compression milling are usually done. Size reduction may provide better results, but very size reduction to fine particle size may impose adverse effects on subsequent processing, such as pretreatment and enzymatic hydrolysis.
- **Pyrolysis:** Pyrolysis is an endothermic process where less input of energy is required. In this process, the materials are treated at a temperature greater than 300 °C to rapidly decompose cellulose to produce gaseous products such as H₂, CO, and residual char. The residual char is further treated by leaching with water or mild acid. The water leachate contains enough carbon sources to support microbial growth for bioethanol production. Glucose is the main component of water leachate. An average of 55% of the total weight of biomass is lost during water leaching (Das *et al.*, 2004).

2. Physicochemical Pretreatment

- Steam Explosion or Autohydrolysis: Steam explosion is a promising method of pretreatment to make biomass more accessible to cellulase attack (Neves *et al.*, 2007). This pretreatment method without any catalyst is promising, and the biomass fractionates to yield levulinic acid, xylitol, and alcohol. In this method, the biomass is heated using high-pressure steam (20-50 bar, 160-290 °C) for a few minutes; the reaction is then stopped by sudden decompression to atmospheric pressure (Sanchez and Cardona, 2008). Steam expansion within the lignocellulosic matrix separates the individual fibers (Balat *et al.*, 2008).
- Liquid Hot Water Method: This method uses compressed hot liquid water at a pressure above the saturation point to hydrolyze the hemicellulose (Neves *et al.*, 2007). The hydrothermal pretreatment method releases a high fraction of hemicellulosic sugars as oligomers. The treatment generally occurs at temperatures of 170-230 °C and pressures above 5 MPa for 20 min.
- Ammonia Fiber Explosion: Ammonia fiber explosion (AFEX) pretreatment involves liquid ammonia and steam explosion (Balat *et al.*, 2008). AFEX is an alkaline thermal pretreatment that exposes the lignocellulosic materials to high temperature and pressure treatment followed by rapid pressure release.
- **CO₂ Explosion:** CO₂ explosion acts in a manner like that of the steam and ammonia explosion techniques. Conversion yields are higher compared to the steam explosion method (Hamelinck *et al.*, 2005). However, a CO₂ explosion is more cost-effective than an ammonia explosion and does not cause the formation of inhibitors as in a steam explosion (Prasad *et al.*, 2007).

- **3.** Chemical Pretreatment: This method involves the usage of dilute acid, alkali, ammonia, organic solvent, SO₂, CO₂, or other chemicals. These methods are easy to operate and have good conversion yields in a short period.
 - Acid Pretreatment: Acid pretreatment is considered one of the most essential techniques and aims for high yields of sugars from lignocellulosic material (Sain, 2020). It is usually carried out by concentrated or diluted acids (usually between 0.2 % and 2.5 % w/w) at temperatures between 130 °C and 210 °C. Moiser *et al.* (2005) reported higher hydrolysis yield from lignocellulose pretreated with diluted H₂SO₄ than other acids. Sulfuric acid is widely used for acid pretreatment among various acid types (Cardona et al., 2009). When wheat straw was treated with 0.75% v/v H₂SO₄ at 121 oC for 1 hour, a saccharification yield of 74% was obtained (Saha *et al.*, 2005).
 - Alkaline Pretreatment: Alkali treatment disrupts the cell wall's lignocellulose by dissolving hemicelluloses, lignin, and silica, hydrolyzing uronic and acetic esters, and swelling cellulose. The crystallinity of cellulose is decreased due to swelling. Pretreatment with alkali digests the lignin matrix and makes hemicellulose and cellulose available for enzymatic degradation (Pandey *et al.*, 2000a). NaOH will boost hardwood digestibility from 14% to 55% by lowering the lignin level from 24-55% to 20% (Kumar and Wyman, 2009).
 - Wet Oxidation: In wet oxidation, the feedstock material is treated with water and either by air or oxygen at temperatures above 120 °C (Martin *et al.*, 2007). The water is added to the biomass at 1 L per 6 g of biomass.
 - Organosolv Pretreatment: An organic solvent or organosolv pulping processes are alternative methods for the delignification of lignocellulosic materials. The utilization of organic solvent/water mixtures eliminates the need to burn the liquor and allows the isolation of the lignins (by distillation of the organic solvent). Examples of such pretreatments include using 90 % formic acid and pressurized carbon dioxide in combination (50 % alcohol/water mixture and 50 % carbon dioxide). Other various organic solvents which can be used for delignification are methanol, ethanol, acetic acid, performic acid and preacetic acid, acetone, etc. (Zhao *et al.*, 2009). A combination of ammonia and ionic liquid pretreatments of rice straw resulted in a 97 % conversion of cellulose to glucose (Nguyen *et al.*, 2010).
- 4. **Biological Pretreatment:** Biological pretreatments are more environmentally friendly than other pretreatment methods because they do not use chemicals, require little energy, do not produce waste streams or problems with corrosion, and produce the least amount of inhibitors. In this method, microorganisms like brown, white, and soft rot fungi break down lignin and hemicellulose (Sanchez, 2009).
 - Enzymes Hydrolysis: Enzymatic hydrolysis includes the use of enzymes for the hydrolysis of raw lignocellulosic materials. Cellulases facilitate the enzymatic breakdown of cellulose so that yeast or bacteria can ferment its reducing sugars to ethanol. (Sun and Cheng, 2002). During the cellulose hydrolysis process, three primary categories of cellulases are involved: endoglucanases, which target less

crystalline areas within the cellulose structure to generate unbound chain ends; exoglucanases/cellobiohydrolases, which further break down the molecule by eliminating cellobiose units from these unbound chain ends; and β -glucosidases, which catalyze the conversion of cellobiose into glucose (Prasad *et al.*, 2007).

Enzymatic Hydrolysis Can Be Divided into Two Stages: Primary and Secondary

- Primary Hydrolysis: The stage involves the action of endoglucanases and exoglucanases on a solid substrate's surface, releasing oligosaccharides (up to 6 glucose units in the chain) into the liquid phase.
- Secondary Hydrolysis: The stage includes further hydrolysis of oligosaccharides to cellobiose (by cellobiohydrolase) and glucose (by β -glucosidases).

Laccase (benzenediol oxygen oxidoreductase), which is synthesized by a variety of white rot fungi, can also be used in lignin degradation (Binod et al., 2010). Lignin is closely bound to cellulose, making it inaccessible to cellulases. So the primary mechanism for breaking down lignin involves peroxidases. Lignin peroxidase /ligninase and manganese peroxidase /Mn-dependent peroxidase are the most important peroxidase enzymes. Laccase (benzenediol oxygen oxidoreductase), synthesized by various types of white rot fungi, can also be used (Binod *et al.*, 2010).

Enzymatic hydrolysis is more environmentally friendly than acid hydrolysis of lignocellulose because it is highly selective and takes place under milder reaction conditions (*at* pH=5 and temperatures below 50 °C). Additionally, it produces more glucose with less byproduct production, which is advantageous for using hydrolysate in fermentation later on.

V. BIOETHANOL PRODUCTION PROCESS

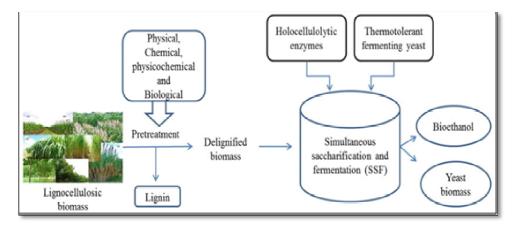
Bioethanol can be produced from a large variety of carbohydrates (mono, di, and polysaccharides). Polysaccharides are often organised in chains of bonded monosaccharides, which result from dehydration syntheses. Polysaccharides and disaccharides are usually broken down into monosaccharides, and later, monosaccharides are converted to bioethanol and CO₂.

Yeast fermentation is a well-established natural metabolic process where industrial yeast strains turn complex carbohydrates into single sugars and sugar into an alcohol or an acid. Usually, two reactions convert cellulose into bioethanol by enzymatic hydrolysis and fermentation process.

 $\begin{array}{cccc} C_{12}H_{22}O_{11} + H_2O & & & \\ Sucrose & Water & & \\ C_6H_{12}O_6 & & & \\ Glucose & or Fructose & & \\ C_6H_{12}O_6 & & & \\$

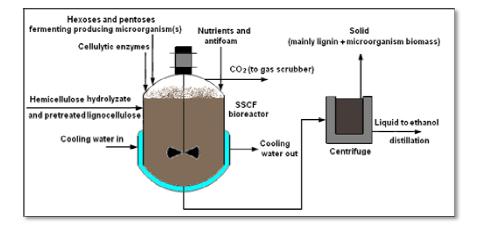
Bioethanol production largely depends on fermentation processes which are,

- Simultaneous saccharification and fermentation (SSF)
- Simultaneous saccharification and co-fermentation (SSCF)
- Separate hydrolysis and fermentation (SHF)
- Solid-state fermentation (SSF)
- 1. Simultaneous Saccharification and Fermentatio(SSF): Simultaneous saccharification and fermentation (SSF) is a process that combines enzymatic hydrolysis with fermentation to obtain value-added products in a single step. This process uses an enzymatic complex to hydrolyze cellulose and obtain sugars. These sugars are later used by microorganisms and are converted into value-added products. SSF has several advantages over other fermentative processes. Some of the advantages compared to separate enzymatic hydrolysis and fermentation (SHF) are the use of a single vessel for the fermentation and saccharification, reducing both residence times and the capital costs of the process, and the reduction of inhibitory compounds from enzymatic hydrolysis, which improves the overall performance of the process. Due to these advantages, SSF has been widely investigated to produce biofuels such as ethanol and butanol from lignocellulosic and starchy raw materials (Das-Neves et al., 2007).
 - Among the disadvantages are the pH and temperature of the process since the optimum temperature of enzymatic hydrolysis is typically more significant than the fermentation temperature. Therefore, it is necessary to find an equilibrium point where the process works appropriately (Niphadkar *et al.*, 2017)

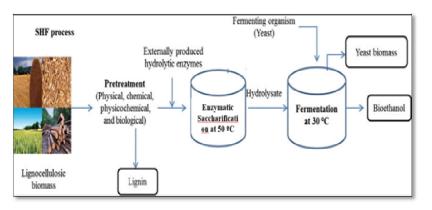


2. Simultaneous Saccharification and Co-Fermentation (SSCF) : Simultaneous saccharification and co-fermentation (SSCF) is another alternate process to SSF, which simultaneously allows hexose and pentose fermentation. In SSCF configuration, microorganisms used for fermentation should have similar operating pH and temperature. SSCF offers the potential of streamlined processing while reducing capital costs (Cardona and Sanchez, 2007).

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3. Separate Hydrolysis and Fermentation (SHF): The separate hydrolysis and fermentation (SHF) process has been implemented for ethanol production and is a starchbased ethanol production process. In this process, starch is initially catalysed by the action of amylolytic enzymes, *viz*. α -amylase (for liquefaction) and glucoamylase (for saccharification). The process can be accomplished by fermentation in separate vessels. The major disadvantage of this process is inhibiting enzyme activity due to the accumulation of hydrolysed sugar. It is also an expensive and time-consuming process (Das-Neves *et al.*, 2007).



4. Solid State Fermentation (SSF): Solid-state fermentation is an efficient, cost-effective, and promising technology in which microorganisms grow on the surface of solid materials in the absence of free water resulting in the elimination of the sugar extraction process and less water production, which in turn yields lower distillation and purification costs. Furthermore, SSF is a well-established technology for the production of different enzymes. This potential of SSF makes it an appropriate process for enzymatic pretreatment and hydrolysis of substrates and subsequent bioethanol production. (Pandey *et al.*, 2000b).

VI. FERMENTATION MODES

1. Batch Fermentation: A set amount of medium, which includes nutrients and other components, is given to microorganisms. As people ingest nutrients, the culture and environment are continuously changing.

Advantages	Disadvantages		
 Inexpensive Low danger of contamination Less need for control Simpler sterilization 	 Ethanol production with fewer cells per cell increased downtime between batches because of vessel setup, cleaning, and sterilization 		

2. Fed-Batch Fermentation: Microorganisms are introduced to the media by inoculation, and after growing in a batch environment for a predetermined period of time, nutrients are gradually added during the fermentation (Yang and Sha, 2019).

Advantages	Disadvantages
Higher ethanol accumulation	• Rising expenses for process
Limited by-product accumulation	control
• Maintenance of maximum viable cell concentration	• Increased downtime between batches because of cleaning, vessel
Increased cell lifespan	setup, and sterilization
• Control of variables like pH,	
temperature, and dissolved oxygen	

3. Continuous Fermentation: The nutrients consumed are continuously replaced in the fermenter with a fresh medium. The removal of hazardous metabolites, used media, and ethanol is ongoing (Yang and Sha, 2019).

Advantages	Disadvantages			
 Reduced vessel cleaning downtime Increased productivity Lower costs; greater degree of control Ability to automate 	 Long development times can raise the danger of contamination Cell aggregation can inhibit optimal steady-state growth. 			
More cost-effectiveLess prone to human error.				

Table 2: Difference between Batch, Fed Batch, and Continuous Fermentation

Characteristics	Batch Fed batch		Continuous	
Cultivation system	Closed type	Semi-closed type	Open type	
Addition of fresh nutrition	No	Yes	Yes	
Volume of culture	Constant	Increases	Constant	
Removal of	No	No	Yes	

wastes				
Chance of contamination	Minimum	Intermediate	Maximum	
Growth phase	Lag, Log, Stationary and Decline phase	Lag, Log, Stationary and Decline phase	Lag and Log phase	
Log phase	Shorter	Longer	Longest and continuous	
Product yield Low		Medium	High	

VII. BIOETHANOL SEPARATION AND PURIFICATION

To produce pure ethanol (95.63% by mass) from binary azeotrope ethanol-water, two energy-intensive separation stages are required (Huang et al., 2008). In the first phase, ethanol is concentrated to a mass concentration of between 92.4 and 94% by conventional distillation. An energy-efficient option with relatively moderate investments is cyclic distillation for the purification of ethanol.

The process of ethanol dehydration is used in the second phase to create anhydrous ethanol, which has ethanol concentrations higher than those seen in azeotropic composition. That can be accomplished using a number of well-known techniques, including pressureswing distillation (Mulia-Soto and Flores-Tlacuahuac, 2011), extractive distillation (with liquid solvent, dissolved salt, their mixture, ionic liquids, and hyperbranched polymers), azeotropic distillation, and combinations of these techniques.

Membrane techniques have attracted attention as an alternative to conventional distillation because of a variety of benefits that make them appealing for the separation of liquid mixtures. They can be used to separate temperature-sensitive materials because of their excellent separation efficiency, cheap energy and operating costs, lack of waste streams, and ability to operate at low temperatures (Radocaj and Diosady, 2014).

Parallel to fermentation, pervaporation is a possibility. This technique, which is safe for the working microorganism and allows for in-situ ethanol extraction, shows promise (Kaewkannetra et al., 2011). For the extraction of ethanol and other volatile components from fermentation broth, gas stripping is an additional method in addition to distillation (de Vrije et al., 2013).

According to Marjani et al. (2014), ethanol separation also used the carbon nanotubeintegrated polyamide nanocomposite membrane. The silicalite-1, polydimethylsiloxane, and polyvinylidene fluoride hybrid composite membrane were used in the fermentation of sorghum juice and continuous bioprocess (Cai et al., 2016).

1. Fourth-generation bioethanol (4G): Compared to wild-type algae, genetically modified algae can produce more products and have a number of other advantages. CRISPR/Cas9

is a commonly utilized method in genetic engineering because it combines an easy design with effective transfection and targeted gene disruption.

The successful manufacture of ethanol as well as other fuel products, including butanol, isobutanol, and modified fatty acids, has been achieved in fourth-generation biofuel methods that depend on genetically enhanced Cyanobacteria.



Asia's First 2G Ethanol Bio-Refinery of Indian Oil Corporation Limited (IOCL) at Panipat, Haryana (10th Aug 2022).

2. Advantages of Bioethanol

- Encourages farm income
- Carbon neutral
- Reduce energy costs
- Replaces the usage of high price petroleum

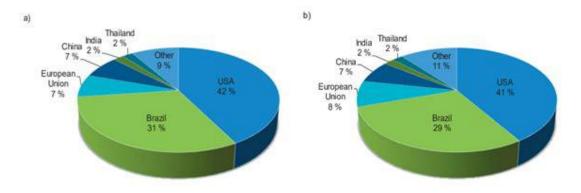
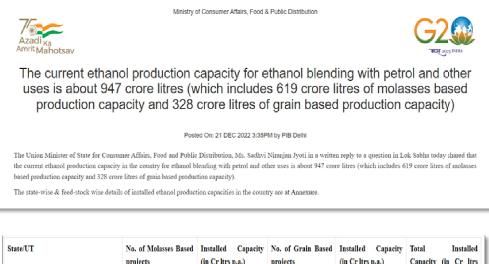
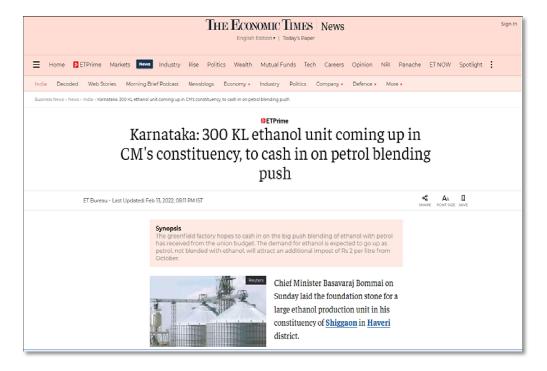


Figure 12: Predictions of the World Bioethanol (a) Production and (b) Consumption by 2024



State/UT	No. of Molasses Based projects	Installed Capacity (in Cr ltrs p.a.)	No. of Grain Based projects	Installed Capacity (in Cr ltrs p.a.)	Total Installed Capacity (in Cr ltrs p.a.)
Karnataka	33	100.2	6	18.5	118.7

https://pib.gov.in/PressReleasePage.aspx?PRID=1885392



3. Ministry of Petroleum & Natural Gas

India has achieved the target of 10 percent ethanol blending, 5 months ahead of schedule. Posted On: 05 JUN 2022 2:11PM by PIB Delhi

A "Roadmap for Ethanol Blending in India 2020-25" was released by the Hon'ble Prime Minister in June 2021, which lays out a detailed pathway for achieving 20 percent

ethanol blending. This roadmap also mentioned an intermediate milestone of **10 percent** blending to be achieved by November 2022. Twelve commercial plants have already been proposed to be built under the Pradhan Mantri JI-VAN (Jaiv Indhan-Vatavaran Anukool Fasal Awashesh Nivaran) Yojana in regions with adequate biomass supply.





Companies

- Balrampur Chini Mills Ltd.
- Triveni Engineering Ind.
- Shree Renuka Sugars Ltd.
- EID Parry (India) Ltd
- Bajaj Hindusthan Sugar Company
- Godavari Biorefineries Limited
- Dalmia Bharat Sugar and Industries Limited
- Simbhaoli Sugars Limited

4. Uses of Bioethanol has Several Applications, Including

- Uses of Fuel: It is blended with gasoline to create ethanol fuel (commonly known as E10 or E85, depending on the ethanol-to-gasoline ratio). Ethanol is used as a renewable and cleaner-burning alternative to fossil fuels, reducing greenhouse gas emissions and dependence on finite resources.
- **Industrial Applications:** Ethanol is used as a solvent in the manufacturing of various products, including pharmaceuticals, personal care items, and cleaning agents.
- Alcoholic Beverages: Ethanol is the primary alcohol found in alcoholic beverages.
 - Benefits and Challenges: Bioethanol offers several environmental benefits, as it is produced from renewable resources and reduces net carbon dioxide emissions compared to fossil fuels. However, there are also challenges associated with its production, including competition with food crops, land-use changes, and potential impacts on water resources.

Researchers and scientists continue to work on improving bioethanol production methods and exploring alternative feedstocks that are less resource-intensive and do not directly compete with food production. Additionally, advancements in second-generation biofuels, such as cellulosic ethanol, aim to use non-food-based feedstocks like agricultural residues and municipal solid waste, further reducing environmental impacts.

Overall, bioethanol remains an essential component of the global effort to transition to more sustainable and environmentally friendly energy sources.

VIII. CONCLUSION

India produces roughly 350 million tonnes of agricultural waste annually. Uncontrolled agricultural solid waste disposal and burning has led to pollution, a threat to human life, and other environmental issues. Through a variety of uses, including the manufacture of fuel, fertilizers, animal feed, etc., these wastes can be managed effectively. The idea of turning agricultural waste into bioethanol will help keep the environment clean, and the production of bioenergy will help us overcome the problems associated with the depletion of fossil fuels. The environmentally friendly bioethanol made from agricultural waste is a valuable replacement for fossil fuels. The burning of conventional gasoline with a small amount of bioethanol produces less greenhouse gas emissions. Modern automobiles can also run flawlessly on bioethanol blends without modifying their engines, which has improved environmental sustainability and reduced energy use. The need for sustainable energy sources to reduce dependency on imported petroleum oil means that bioethanol is a potential alternative energy source in the future.

- Eco-friendly alternative to non-renewable fuels
- Clean environment
- Reduces greenhouse gas emissions during combustion
- Modern motor vehicles run perfectly on bioethanol blends without any engine modification.

REFERENCES

- [1] [Anonymous]. 2020. Creating Wealth From Agricultural Waste. Ministry of Agriculture and Farmers Welfare, Government of India, 148p.
- [2] Anoop, P. 2014. Utilization of pineapple (*Ananas comosus* (L.) Merr.) Biomass for biofuel production. M.Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 103p.
- [3] Arifin, Y., Tanudjaja, E., Dimyati, A., and Pinontoan, R. 2014. A second generation biofuel from cellulosic agricultural by-product fermentation using clostridium species for electricity generation. *Energy Procedia* 47: 310–315.
- [4] Balat, M., Balat, H., and Oz, C. 2008. Progress in bioethanol processing. Prog. Energy Combust. Sci. 34: 551-573.
- [5] Ballesteros, I., Negro, M. J., Oliva, J. M., Cabanas, A., Manzanares, P., and Ballesteros, M. 2006. Ethanol production from steam-explosion pretreated wheat straw. *App. Biochem. Biotechnol*. 130: 496-508.
- [6] Binod, P., Sindhu, R., Singhania, R. R., Vikram, S., Devi, L., and Nagalakshmi, S. 2010. Bioethanol production from rice straw: An overview. *Bioresour. Technol.* 101(13): 4767–4774.
- [7] Borines, M. G., de Leon, R. L., and Cuello, J. L. 2013. Bioethanol production from the macroalgae *Sargassum* spp. *Bioresour. Technol.* 138: 22–29.
- [8] Cai, D., Hu, S., Chen, C., Wang, Y., Zhang, C., and Miao, Q. 2016. Immobilized ethanol fermentation coupled to pervaporation with silicalite-1/polydimethylsiloxane/polyvinylidene fluoride composite membrane. *Bioresour. Technol.* 220: 124–131.
- [9] Cardona, C. A. and Sanchez, O. J. 2007. Fuel ethanol production: Process design:trends and integration opportunities. *Bioresour. Technol.* 98(12): 2415-2457.
- [10] Cardona, C. A., Quintero, J. A., and Paz, I. C. 2009. Production of bioethanol from sugarcane bagasse: status and perspectives. *Bioresour. Technol.* 101(13): 4754-4766.

- [11] Casabar, J. T., Unpaprom, Y., and Ramaraj, R. 2019. Fermentation of pineapple fruit peel wastes for bioethanol production. *Biomass Conv. Bioref.* 15: 56-65.
- [12] Chaudhary, L., Pradhan, P., and Soni, N. 2014. Algae as a feedstock for bioethanol production: new entrance in biofuel world. Int. J. Chem. Tech. Res. 6(2): 1381–1389.
- [13] Corbin, K. R., Byrt, C. S., Bauer, S., DeBolt, S., Chambers, D., Holtum, J. A. M., Karem, G., Henderson, M., Lahnstein, J., and Beahan, C. T. 2015. Prospecting for energy-rich renewable raw materials: Agave leaf case study. *PLoS ONE* 10: 135382.
- [14] Danmaliki, G. I., Muhammad, A. M., Shamsuddeen, A. A., and Usman, B. J. 2016. Bioethanol production from banana peels. *IOSR J. Environ. Sci., Toxicol. Food Technol.* 10 (6): 56-62.
- [15] Das, P., Ganesha, A., and Wangikar, P. 2004. Influence of pretreatment for deashing of sugarcane bagasse on pyrolysis products. *Biomass Bioenerg*. 27: 445-457.
- [16] Das-Neves, M. A., Toshinori, K., Naoto, S., and Nakajima. 2007. State of the art and future trends of bioethanol production. Dyn. Biochem. Process Biotechnol. Mol. Boil. 1(1): 1-14.
- [17] de Vrije, T., Budde, M., Vander, W. H., Claassen, P. A. M., and Lopez-Contreras, A. M. 2013. 'In situ' removal of isopropanol, butanol and ethanol from fermentation broth by gas stripping. *Bioresour. Technol.* 137: 153–159.
- [18] Demirbas, A. 2005. Bioethanol from cellulosic materials: a renewable motor fuel from biomass. *Energy Sources* 27: 327-333.
- [19] Dias, M. O. S., Cavalett, O., and Filhob, R. M. 2014. Integrated first and second generation ethanol production from sugarcane. *Chem. Engg. Transac.* 37: 445–450.
- [20] Doda, S. and Sahu, O. 2021. Production of bioethanol from biomass (Marigold flower). *Materials Today: Proceedings.* www.elsevier.com/locate/matpr. 1-6.
- [21] FAO (Food and Agriculture Organization) 2008. The State of Food and Agriculture 2008. Biofuels: Prospects, Risks and Opportunities. Available online: http://www.fao.org/3/i0100e/i0100e.pdf (accessed on 5 August 2021).
- [22] Goel, S., Kumar, P., Sain, M., & Singh, A. (2022). Anaerobic Treatment of Food Processing Wastes and Agricultural Effluents. In *Food Processing Waste and Utilization* (pp. 313-328). CRC Press.
- [23] Gomez, L. D., Clare, G. S., and McQueen-Mason, J. 2008. Sustainable liquid biofuels from biomass: the writing's on the walls. *New Phytol.* 178: 473–485.
- [24] Gunam, I. B. W., Dewi, I. A. P. K., Antara, N. S., Anggreni, A. A. M. D., and Setiyo, Y. 2021. Bioethanol production from sugarcane bagasse by *Saccharomyces cerevisiae* ATCC 9763 immobilized in Naalginate. *IOP Conf. Series: Earth Environ. Sci.* 824: 012054.
- [25] Hamelinck, C. N., Hooijdonk, G. V., and Faaij, A. P. C. 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass Bioenerg*. 28: 384-410.
- [26] Huang, H. J., Ramaswamy, S., Tschirner, U. W., and Ramarao, B. V. 2008. A review of separation technologies in current and future biorefineries. *Sep. Purif. Technol.* 62(1): 1–21.
- [27] Kaewkannetra, P., Chutinate, N., Moonamart, S., Kamsan, T., and Chiu, T. Y. 2011. Separation of ethanol from ethanol-water mixture and fermented sweet sorghum juice using pervaporation membrane reactor. *Desalination*. 271(1-3): 88–91.
- [28] Karimi, K., Shafiei, M., and Kumar, R. 2013. Progress in physical and chemical pretreatment of lignocellulosic biomass. In: Biofuel Technologies Recent Developments. Berlin Heidelberg: Springer-Verlag; p. 53–96.
- [29] Khoshkho, S. M., Mahdavian, M., Karimi, F., Karimi-Maleh, H., and Razaghi, P. 2022. Production of bioethanol from carrot pulp in the presence of *Saccharomyces cerevisiae* and beet molasses inoculum; A biomass based investigation. *Chemosphere* 286: 131688.
- [30] Kumar, R. and Wyman, C. E. 2009. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnol. Prog.* 25: 302-314.
- [31] Lennartsson, P. R., Erlandsson, P., and Taherzadeh, M. J. 2014. Integration of the first- and secondgeneration bioethanol processes and the importance of by-products. *Bioresour. Technol.* 165: 3–8.
- [32] Marjani, A., Mohammadi, M., Pelalak, R., and Moradi, S. 2014. Ethanol purification using polyamidecarbon nanotube composite membranes. *Polym. Eng. Sci.* 54(4): 961–968.
- [33] Martin, C., Klinke, H. B., and Thomsen, A. B. 2007. Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse. *Enzyme Microb. Technol.* 40: 426-432.
- [34] Mitchell, D.A. and Lonsane, B. K. 1990. Definition, characterization and economic evaluation. In: Doelle, H. W. and Rolz, C. (Eds.). *General Principles of Solid Substrate Fermentation*. Rapid Publications of Oxford Ltd, UK.
- [35] Mosier, N., Hendrickson, R., Ho, N., Sedlak, M., and Ladisch, M. R. 2005. Optimization of pH controlled liquid hot water pretreatment of corn stover. *Bioresour. Technol.* 96: 1986-1993.

- [36] Mulia-Soto, J. F. and Flores-Tlacuahuac, A. 2011. Modeling, simulation and control of an internally heat integrated pressure-swing distillation process for bioethanol separation. *Comput. Chem. Eng.* 35(8): 1532– 1546.
- [37] Mussatto, S. I., Dragone, G., Guimaraes, P. M. R., Silva, J. P. A., Carneiro, L. M., and Roberto, I. C. 2010. Technological trends, global market, and challenges of bio-ethanol production. *Biotechnol. Adv.* 28(6): 817–830.
- [38] Nahak, S., Nahak, G., and Pradhan, I. 2011. Bioethanol from marine algae: a solution to global warming problem. J. Appl. Environ. Biol. Sci. 1(4): 74–80.
- [39] Neves, M. A., Kimura, T., Shimizu, N., and Nakajima, M. 2007. State of the art and future trends of bioethanol production, dynamic biochemistry, process biotechnology and molecular biology. Global Science Books; 1-13.
- [40] Nguyen, T. A. D., Kim, K. R., Han, S. J., Cho, H. Y., Kim, J. W., and Park, S. M. 2010. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. *Bioresour. Technol.* 101(19): 7432-7438.
- [41] Niphadkar, S., Bagade, P., and Ahmed, S. 2017. Bioethanol production: insight into past, present and future perspectives. *Biofuels*. <u>https://doi.org/10.1080/17597269.2017</u>. 1334338.
- [42] Nugrahini, A. D., Kurniawan, M. P., and Kinasih, D. A. 2021. Development of lignocellulose-based bioethanol from chrysanthemum flower waste (*Chrysanthemum sp.*). *IOP conf. series: Earth and Environ. sci.* 963: 012017.
- [43] Palacios-Bereche, R., Mosqueira-Salazar, K. J., and Modesto, M. 2013. Exergetic analysis of the integrated first- and second-generation ethanol production from sugarcane. *Energy* 62: 46–61.
- [44] Panda, S. K., Ray, R. C., Mishra, S. S., and Kayitesi, E. 2017. Microbial processing of fruit and vegetable wastes into potential biocommodities: a review. *Crit. Rev. Biotechnol.* 1549-7801.
- [45] Pandey, A., Soccol, C. R., Nigam, P., and Soccol, V. T. 2000a. Biotechnological potential of agroindustrial residues. I: sugarcane baggase. *Bioresour. Technol.* 74: 69-80.
- [46] Pandey, A., Soccol, C. R., and Mitchell, D. A. 2000b. New developments in solid-state fermentation. I. Bioprocesses and products. *Process Biochem.* 35 (10): 1153-1169.
- [47] Phuong, D. V., Quoc, L. P. T., Tan, P. V., and Duy, L. N. D. 2019. Production of bioethanol from Robusta coffee pulp (*Coffea robusta* L.) in Vietnam. *Foods Raw Mater*. 7(1): 10-17.
- [48] Phwan, C. K., Chew, K. W., Sebayang, A. H., Ong, H. C., Ling, T. C., Malek, M. A., Yeek-Chia, H., and Show, P. K. 2019. Effects of acids pre-treatment on the microbial fermentation process for bioethanol production from microalgae. *Biotechnol. Biofuels* 12:191.
- [49] Prasad, S., Singh, A., and Joshi, H. C. 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Resour. Conser. Recycl.* 50: 1-39.
- [50] Radocaj, O. and Diosady, L. L. 2014. Continuous ethanol fermentation in immersed, cross-flow microfiltration membrane bioreactor with cell retention. J. Basic Appl. Sci. 10: 543–553.
- [51] Ramachandra, T. V. and Hebbale, D. 2020. Bioethanol from macroalgae: Prospects and challenges. *Renew. Sustain. Energy Rev.* 117: 109147.
- [52] Rodriguez, L. A., Toro, M. E., Vazquez, F., Correa-Daneri, M. L., Gouiric, S. C., and Vallejo, M. D. 2010. Bioethanol production from grape and sugar beet pomaces by solid-state fermentation. *Int. J. Hydrogen Energy* 35: 5914-5917.
- [53] Rodsrud, G., Lersch, M., and Sjode, A. 2012. History and future of world's most advanced biorefinery in operation. *Biomass Bioenrg*. 46: 46–59.
- [54] Saha, B. C., Iten, L. B., Cotta, M. A., and Wu, Y. V. 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochem*. 40: 3693-3700.
- [55] Sain, M. (2020). Production of bioplastics and sustainable packaging materials from rice straw to eradicate stubble burning: A Mini-Review. *Environment Conservation Journal*, 21(3), 1-5.
- [56] Sanchez, C. 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnol. Adv.* 27(2): 185–194.
- [57] Sanchez, O. J. and Cardona, C. A. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour. Technol.* 99: 5270-5295.
- [58] Sarkar, N., Ghosh, S. K., and Bannerjee, S. 2012. Bioethanol production from agricultural wastes: An overview. *Renew. Energ.* 37(1): 19–27.
- [59] Seong, Choi., Jae-Hoon, Kim., Seung, G. W., Kyoung, H. K., and Hyeun-Jong, B. 2013. Bioethanol production from mandarin (Citrus unshiu) peel waste using popping pretreatment. *Appl. Energy* 102: 204– 210.
- [60] Stichnothe, H. and Azapagic, A. 2009. Bioethanol from Waste: Life Cycle Estimation of the Greenhouse Gas Saving Potential. *Resour. Conserv. Recy.* 53: 624–630.

- [61] Sun, R. C., Lawther, J. M., and Banks, W. B. 1995. Influence of alkaline pretreatments on the cell-wall components of wheat-straw. *Ind. Crops Prod.* 4(2): 127-145.
- [62] Sun, Y. and Cheng, J. 2002. Hydrolysis of lignocellulosic material for ethanol production: a review. *Bioresour. Technol.* 96: 673-686.
- [63] Taha, M. G., Khattab, A. E., Ali, H. E., and Dawood, M. A. M. 2019. Production of bio-ethanol from potato starch wastes by *Saccharomyces cerevisiae*. *Egypt. J. Appl. Sci.* 34 (12): 256-267.
- [64] Tomas-Pejo, E., Alvira, P., Ballesteros, M., and Negro, M. J. 2011. Pretreatment technologies for lignocellulose-to-bioethanol conversion. In: Pandey A, Larroche C, Ricke SC, Dussap CG, Gnansounou E, editors. Biofuels – Alternative feedstocks and conversion processes. Oxford, UK: Academic Press; 2011. pp. 149–176.
- [65] Uihlein, A. and Schbek, L. 2009. Environmental impacts of a lignocellulosic feedstock biorefinery system: an assessment. *Biomass and Bioenergy* 33: 793-802.
- [66] Yang, Y. and Sha, M. A. 2019. Beginner's Guide to Bioprocess Modes—Batch, Fed-Batch, and Continuous Fermentation; Eppendorf Application Note 408; Eppendorf: Hamburg, Germany.
- [67] Zhao, X., Cheng, K., and Liu, D. 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* 82: 815-827.