

# Advancing 2G Ethanol: Technologies, Pathways, and Sustainability in Modern Biorefineries

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## Abstract

As the global energy landscape shifts toward decarbonization, the transition from first-generation (1G) to second-generation (2G) biorefineries has become essential to ensure energy security without compromising food supplies. This review examines the architectural evolution of biomass biorefineries, contrasting the established 1G starch-and-sugar-based models with the emerging 2G lignocellulosic frameworks. We provide a detailed technical analysis of the three critical stages of 2G ethanol production: pretreatment (to overcome biomass recalcitrance), hydrolysis (to liberate fermentable sugars), and fermentation (optimizing yeast performance for pentose and hexose sugars). Furthermore, the article evaluates the scope of 2G biorefineries in India, highlighting the nation's ambitious E20 blending targets and the role of agricultural residues in reducing carbon footprints and stubble burning. By analyzing current technological bottlenecks and policy incentives, this review outlines the potential for 2G ethanol to act as a cornerstone of India's sustainable bio-economy.

**KEYWORDS:** Lignocellulosic Biomass, 2G Ethanol, Biorefinery Integration, Sustainable Energy Transition.

## INTRODUCTION

Over the past several decades there have been many significant changes in the use of biomass for raw materials in all markets (food, feed, materials, and energy). The factors include population growth; limited available land for sustainable crop production; and the interest of many countries to move from fossil fuels to more bio-based economies. In order to help each of these markets achieve sustainability and enhanced food security, it is necessary to maximize efficiency across all steps of the biomass-utilization chain from the production of biomass to its conversion into energy, the end product (in this case, fuels), and the disposal or recycling of the remaining biomass. Biorefineries are similar to petroleum-refinery operations in that they strive to minimize the amount of energy expended while maximizing the total use of biomass to manufacture a wide variety of products, thereby meeting the needs of each of the four biomass markets. This makes them essential components in this context. The biomass biorefineries are broadly categorized into various possible biorefinery approaches, as shown in Table 1.

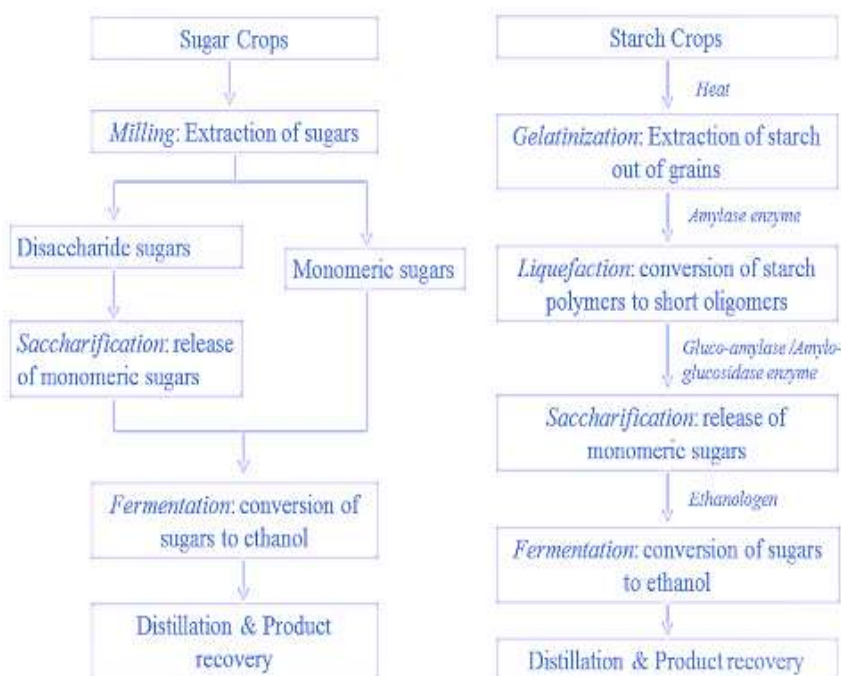
**Table 1 illustrates various biorefinery strategies.**

|                                    |                                                                                                                                                                                                                                                   |
|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Sugar-Based Biorefineries:</b>  | <b>Feedstock:</b> Sugarcane, sugar beets, or other sugar-rich crops.<br><b>Processes:</b> Extraction of sugars for fermentation to produce biofuels (ethanol), biochemicals, and other products.                                                  |
| <b>Starch-Based Biorefineries:</b> | <b>Feedstock:</b> Crops rich in starch, such as corn, wheat, or potatoes.<br><b>Processes:</b> Starch is broken down into sugars through enzymatic processes and then fermented into biofuels (ethanol) or used for other biochemical production. |
| <b>Cellulosic Biorefineries:</b>   | <b>Feedstock:</b> Materials high in cellulose, such as forestry                                                                                                                                                                                   |

|                                       |                                                                                                                                                                                                                                                                                                                                 |
|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                       | <p>waste, agricultural residues, or crops grown specifically for energy.</p> <p><b>Processes:</b> Conversion of cellulose into sugars through various methods like enzymatic hydrolysis or thermochemical processes, followed by fermentation or chemical conversion to produce biofuels, biochemicals, and other products.</p> |
| <b>Lignocellulosic Biorefineries:</b> | <p><b>Feedstock:</b> Biomass containing both cellulose and lignin, such as wood or agricultural residues.</p> <p><b>Processes:</b> Extraction and conversion of cellulose and lignin into different products, including biofuels, bioenergy, and high-value chemicals.</p>                                                      |
| <b>Algae-Based Biorefineries:</b>     | <p><b>Feedstock:</b> Algae species are cultivated for their high lipid content.</p> <p><b>Processes:</b> Extraction of lipids for biodiesel production, as well as the utilization of other components like proteins and carbohydrates for various applications in food, feed, and chemicals.</p>                               |
| <b>Green Biorefineries:</b>           | <p><b>Feedstock:</b> Fresh biomass, such as crops and other plant materials.</p> <p><b>Processes:</b> Processing of fresh biomass to obtain valuable products, including biofuels, bioenergy, and biochemicals, while minimizing waste generation.</p>                                                                          |
| <b>Waste-Based Biorefineries:</b>     | <p><b>Feedstock:</b> Organic waste, agricultural residues, or municipal solid waste.</p> <p><b>Processes:</b> Transformation of waste materials into biofuels, bioenergy, biochemicals, and other useful products that aid in resource usage and waste management.</p>                                                          |

## 1G BIOREFINERIES

The 1G of bioethanol production uses food crops that are abundant in sugar and starch<sup>1</sup>. This is a well-known method that is frequently used to produce bioethanol for commercial use. Bioethanol is produced by fermenting the sugars that are liberated from the ground plants. The enzyme invertase is necessary for the conversion of disaccharides, including sucrose, into monomers<sup>2</sup>. However, in order to create bioethanol, starch needs to be hydrolyzed and gelatinized (Fig.1). According to Mathobo et al. the process of gelatinization entails boiling food in water to cause starch granules to expand and release starch<sup>3</sup>. The starch-rich slurry is liquefied using amylase, an enzyme that breaks down long amylose polymers of starch into short oligosaccharides. The next stage in the saccharification procedure is the change of oligomers into monomeric sugars by glucoamylase and amyloglucosidase treatment. Ultimately, sugar monomers are converted to bioethanol by a microbial strain that produces ethanol. The 1G bioethanol production technique currently in use employs starch or sugar crops to produce bioethanol. More than 70% of global bioethanol is generated in Brazil and the United States, the foremost producers, utilizing sugarcane and corn, respectively. Consequently, the main manufacturers of ethanol, including alcohol fuels, at the moment are big agricultural nations like the United States, Brazil, and China<sup>4</sup>. Ninety-five percent of bioethanol produced in the US is made from maize grain starch<sup>5</sup>.



**Fig 1: Outline of the 1G bioethanol from sugar crops and starch crops**

Bioethanol is usually made from sugarcane juice or molasses, two byproducts of the sugarcane industry. Fresh sugarcane juice accounts for about 79% of Brazil's ethanol production, with cane molasses making up the remaining amount. Cane juice is not currently used in India; instead, sugarcane molasses serves as the main substrate for the synthesis of bioethanol<sup>6</sup>. The solid residue following juice extraction, known as bagasse, has the potential to boost bioethanol production. The amount and duration of time that food crops can be utilized as building blocks for bioethanol production are up for debate. The usage of food crops in the production of 1G bioethanol and an increase in the human population are the causes of this. Moreover, the price of current bioethanol feedstocks could be significantly impacted by this problem. Consequently, non-food materials will be prioritized and will remain the sole sustainable source of renewable feedstock for bioethanol production<sup>7</sup>. 1G feedstocks, such as sugarcane and cereals, are the most often used feedstocks in the industrial-scale production of bioethanol due to their higher quantities of fermentable sugars and total carbs<sup>8</sup>. Growing feedstock crops slows down plant growth and necessitates a lot of arable land, which is a major barrier to the development of bioethanol production. Because of the long-chain polymeric structure of starch, ethanol cannot be directly produced from it.

To break down the polymer into monomers, hydrolysis is required. The bioconversion of starch to ethanol usually requires the following steps: milling, liquefaction, hydrolysis, fermentation, and distillation. Wet milling and drying are the two primary methods used to produce bioethanol from corn. After being processed using a dry milling method, the maize is combined with water to create a slurry. Afterwards, amylopectin and amylase are broken down by adding  $\alpha$ -amylase to the crushed corn slurry. This process converts the sugars into dextrose and maltose. Furthermore, glucoamylase is added, and the mixture is continually agitated at 60 rpm for approximately two hours, hydrolyzing maltose to glucose. The extracted sugar is combined with a fresh strain of yeast and fermented again to produce ethanol. To separate the alcohol portion from the solid fraction, a distillation step is also required. Animals can be given the fraction that is left over after ethanol recovery.

Over 90% of the gasoline ethanol used in automobiles in the US is produced using the dry mill process. Initially, the maize is treated with a diluted sulphur dioxide solution during wet milling, which will dissolve the maize over 24 - 48 hours. After the maize germ is separated from the other components using various grinding

methods (continuous centrifugation, hydrocyclones) the remaining oil has been extracted from the maize. A grit screen is used to separate fiber, gluten, and starch. Finally, the starch-rich part is converted to bioethanol by using techniques similar to the dry milling process<sup>9</sup>. Unlike the bioethanol process based on starch, the procedures involved in producing bioethanol from sugar-based substrates do not include the liquefaction and breakdown stages. Milling is the first step, and it involves cleaning, chopping, and shredding sugarcane. Saccharose sugar is concentrated after milling, juice is extracted with crushers, impurities are eliminated chemically, and sugar is fermented to create bioethanol. The 2G bioethanol refinery produces and uses bagasse, a waste byproduct, as a raw material. The complete method of producing ethanol from sugarcane is referred to as the Melle-Boinot process. After distillation, ethanol is separated, and the non-alcoholic part—known as vinasse—contains a lot of calcium, potassium, and nitrogen, among other nutrients<sup>10</sup>.

## 2G BIOREFINERIES

In the quest for renewable and non-food materials, lignocellulosic material has been suggested as an environmentally friendly feedstock for the generation of bioethanol. Cellulosic biomass has been reported to have a better potential for bioethanol generation than starch and sugar crops<sup>11</sup>. Lignocellulosic components are used to make cellulosic bioethanol, also known as second-generation bioethanol. The most abundant renewable resource on the planet is lignocellulosic cellulose<sup>12</sup>. It is the most important structural component of all crops and accounts for over half of the world's biomass<sup>13</sup>. By 2022, the United States' Energy Independence and Security Act of 2007 aimed to produce 36 billion gallons of renewable fuel, with 16 billion gallons coming from cellulosic bioethanol<sup>14</sup>.

Biomass cannot be converted into bioethanol as easily as starch or sugar can be converted into bioethanol. Converting lignocellulose to bioethanol involves four steps: pretreatment of the biomass followed by saccharification (the conversion of the carbohydrates into sugars), fermentation of the sugars to bioethanol, and recovery of bioethanol from the fermentation broth (product recovery). The pretreatment of the biomass is the hardest step of the ethanol-producing process because lignocellulose comprises cellulose and hemicellulose which are both encapsulated in a very undigestible wall called lignin. Therefore, the purpose of pretreatment is to remove as much lignin from the biomass as possible, exposing the carbohydrates of the lignocellulose for further processing. The pretreatment of the lignocellulosic biomass creates additional complexity and costs to the ethanol production process. Next, the carbohydrate polymers made up of cellulose and hemicellulose, are subsequently hydrolyzed during the saccharification step to form their respective monosaccharides (hexose and pentose). Then, during the fermentation step, the monosaccharides are converted to bioethanol through fermentation by yeasts or other microorganisms.

## PRETREATMENT

The biomass pretreatment, which breaks up the lignocellulosic biomass complex, is the first stage in the 2G bioethanol process. Pretreatment's primary objective is to eliminate lignin and reduce cellulose crystallinity so that the biomass is more accessible to enzymes during the hydrolysis process. Chemical, biological, physicochemical, and physical are the four main types of pretreatments. Reducing the biomass's size and increasing its surface area is the main goal of the physical pretreatment procedure. The processes of grinding, milling, chopping, freezing, and extrusion can be used to provide physical pretreatment. The chemical pretreatment process uses various chemicals, such as alkali, acids, and other organic solvents. The polysaccharides in biomass can be dissolved into monosaccharides by treating them with acid. Alkali pretreatment has caused the internal connections between the lignin and hemicellulose to break down. The combined effects of physical and chemical processes are used in the physicochemical method. It includes liquid hot water pretreatment, ammonia pretreatment, steam explosion, wet oxidation, and carbon dioxide explosion. When high-pressure steam is applied to chipped biomass during the steam explosion process, it changes the structure of the lignin and breaks down the hemicellulose, which makes it easier for the cellulose to break down. Like a steam explosion, an ammonia fiber pretreatment and a carbon dioxide explosion both involve putting biomass under high temperature and pressure with liquid ammonia and CO<sub>2</sub> for short periods of time. This makes the cellulose easier to digest<sup>15</sup>. The method of biological pretreatment involves breaking down the lignin and hemicellulose with the aid of microorganisms or enzymes. This process takes a long time

but uses little energy. Occasionally, in order to achieve the intended outcomes, two or more pretreatment techniques are combined to increase process efficiency.

### HYDROLYSIS

The goal of this process is to convert the cellulose and hemicellulose polymers into sugars. Generally, using an acid or an enzyme can cause hydrolysis. Acid hydrolysis comes in two flavors: concentrated and diluted. While diluted acid hydrolysis employs a low acid concentration at high temperature and pressure for a few seconds to minutes, concentrated acid hydrolysis uses a high acid concentration at low temperature and pressure. In order to produce fermentable sugars, it can break down cellulose and hemicellulose and eliminate lignin. When diluted acid hydrolysis occurs at high temperatures, it produces chemicals that hinder the decomposition of hemicellulose, such as carboxylic acids, furans, phenolic compounds, etc. One disadvantage of this process is the creation of inhibitory compounds by acid hydrolysis, which has an impact on the yield of ethanol. The rusting issue that arose from the use of acid is this technique's second disadvantage<sup>16</sup>.

Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin; these three materials are each broken-down enzymatically into simpler sugars via hydrolysis to valorize for use in many types of products. The cellulose is considered the main component of the biomass and has three primary types of enzymes which are endoglucanase, exocellulase, and  $\beta$ -glucosidase. The endoglucanase has the primary task of breaking down the amorphous (soft) portion of the cellulose structure. The exocellulase has the task of removing cellobiose units from the terminal ends (reducing and non-reducing) of the cellulose polymer. The  $\beta$ -glucosidase will convert cellobiose (two glucose units) into glucose for use by microorganisms such as yeast. Fungi primarily produce the enzymes listed above, however, as hemicellulose contains varying sugar units; hemicellulase is a complement to the above enzymes for hydrolysis of hemicellulose. The exact way that enzymatic saccharification works depends on several things, such as the amount of enzymes used, the time it takes to break down the material, how well the cellulase works, and other structural details. The success of saccharification is contingent upon pretreatment delignification. This process is costly and time-consuming, yet it is non-chemical<sup>17</sup>.

### FERMENTATION

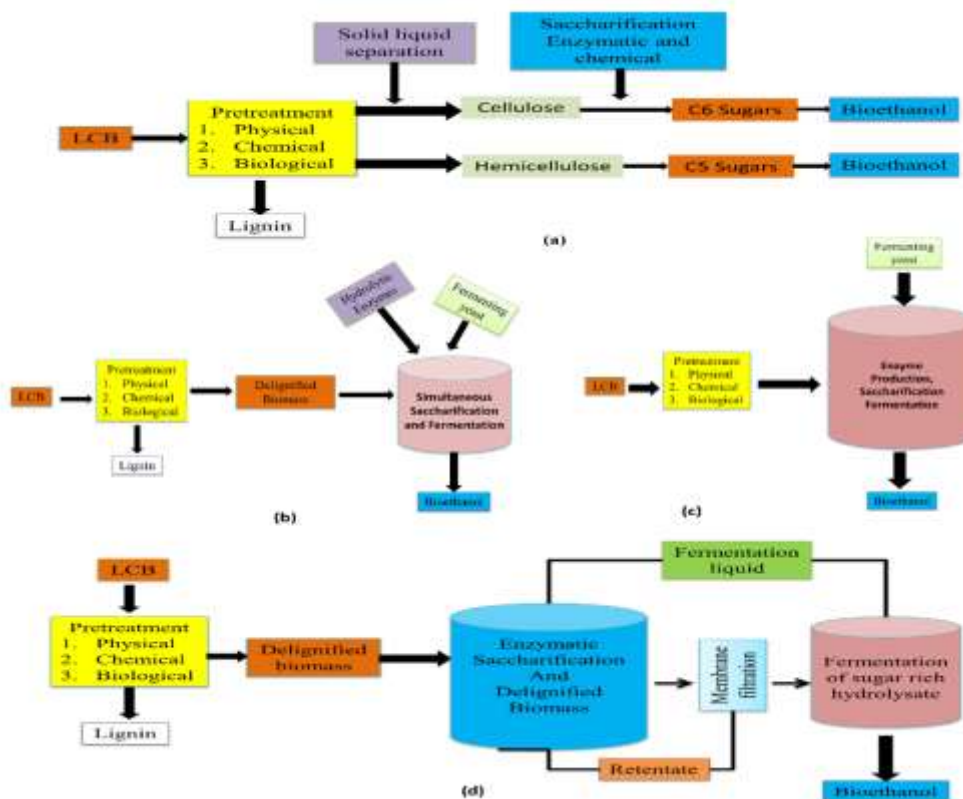
It is a process that uses microorganisms to turn fermentable carbohydrates into bioethanol. Three different configurations are possible: simultaneous saccharification and fermentation (SSF), consolidated bioprocessing (CBP), and separated hydrolysis and fermentation (SHF). Working on both processes' ideal conditions hydrolysis and fermentation is made possible by SHF. However, the main disadvantage of this procedure is the inhibition of the end product, which is glucose buildup. Due to the merger of hydrolysis and fermentation, SSF eliminates the negative effects of sugar aggregation and minimizes the number of processes. However, this technology's downside is the variance in temperature required by fermenting microorganisms and enzymes. The method that combines each phase of the process is called CBP. This technology can have a lower capital cost if the steps are reduced<sup>17</sup>.

Because it offers the greatest flexibility in terms of choosing the hydrolysis process, this approach is the most commonly used one. SHF is a traditional design where the biomass is pretreated, the cellulose is hydrolyzed, and the mixture is then fermented<sup>18</sup>. The insoluble and solid fractions are then separated after the hydrolysate has been neutralized. After that, it is fermented to make alcohol. After being retained, the insoluble fraction is treated with cellulase and glycosidase to release hexose sugar, which is then fermented to produce bioethanol. To provide energy for the entire process, lignin is burned as a leftover insoluble material<sup>19,20</sup>. In this procedure, pretreatment LCB is hydrolyzed to produce sugar monomers using an enzyme that is externally produced. All procedures are done independently since the optimal temperature for fermentation (30–35 °C) and hydrolytic enzymes (approximately 50 °C) are different. The SHF process produced somewhat more ethanol (76.47%) than the SSF process, although it used the same quantity of maize cob biomass and *Trichoderma reesei* enzymes. However, both procedures produced a significant amount of bioethanol (Fig. 2a). The reducing sugars from a study on *G. tenuistipitata* were made by acid hydrolysis and fermented with *S. cerevisiae* to create 0.042 g/g of bioethanol<sup>21</sup>.

The idea of simultaneously performing fermentation and enzymatic hydrolysis was put forth in a patent by Gauss et al.<sup>22</sup>. The enzymatic saccharification of the pretreatment biomass, followed by fermentation of the enzymatic hydrolysate within the same vessel when using this technique, are key difficulties for the

performing. To develop process parameters to allow for simultaneous optimization of the fermentation organisms and the saccharification enzymes; and allowing sufficient time for the saccharifying microorganisms to produce enough enzyme activity to effectively convert the biomass into fermentable sugars. Because the yeast uses the glucose produced during hydrolysis at the same time, it does not build up in the reaction media and reduces the inhibition of cellulase by glucose, cellobiose, and hydrolytic products<sup>23</sup>. Ohgren et al. reported that, compared to the SHF approach, the SSF procedure produced 13% more LC ethanol<sup>24</sup>. This method primarily makes use of thermophilic yeast and cold-adaptive hydrolytic enzymes. It is done in a single jar at about 40 °C in temperature. The most promising of these is *Kluyveromyces marxianus*, because strains of this yeast can grow at temperatures as high as 45–52 °C and can create ethanol at 38–45 °C. The ability to use a range of sugar sources at high temperatures, such as galactose, xylose, arabinose, or mannose, is another advantage of *K. marxianus*<sup>25,19</sup> (Fig. 2b).

Microorganisms that can degrade both the hexose and the pentose in cellulose and hemicellulose must be used in order to produce ethanol at a reasonable cost. All of the sugars released during the hydrolysis and treatment of LCB are completely absorbed into Simultaneous Saccharification and Co-fermentation (SSCF)<sup>26</sup>. It has been proposed to use a mixed culture of yeasts capable of fermenting both C5 and C6 sugars. However, microorganisms are better at turning C6 sugars into ethanol because they grow faster than yeasts that ferment C5 sugars<sup>19</sup>. One of the many great things about the SSCF method is that it constantly gets rid of the byproducts of enzyme hydrolysis that get in the way of cellulases or  $\beta$ -glucosidases. A high water-insoluble solids content helps mix the ingredients and makes more bioethanol (Fig. 2c)<sup>27,28</sup>. Because it allows for the maintenance of lower glucose concentrations than would be maintained by the fermentation of either hexose or pentose sugars, these advantages can ultimately lead to increased efficiency in co-fermenting hexose and pentose carbohydrates. However, the highest dollar-value barriers currently inhibiting the commercialization of biomass-to-ethanol production systems are the costs associated with pretreatment and enzymatic cellulose hydrolysis. For each of the steps in enzyme synthesis, biomass digestion, and glucose fermentation, consolidated bioprocessing uses a single unit operation or reactor to achieve each of these processes simultaneously. For the purpose of converting feedstock into ethanol, consolidated bioprocessing is a process-process fusion (Fig. 2d)<sup>29,30</sup>.



**Fig. 2: Flow of a) SHF, b) SSF, c) SSCF, and d) CBP**

### **SCOPE OF 2G ETHANOL BIOREFINERY IN INDIA**

India is the world's 4<sup>th</sup>-largest producer of greenhouse gases (GHG) and the world's fourth-largest user of petroleum. Because almost 95% of the fuel used for transportation is derived from petroleum products, the nation is largely dependent on imports of petroleum products. Therefore, it is necessary to substitute this significant fraction of transport fuel with renewable energy sources, particularly second generation bioethanol. Furthermore, the nation produces around  $6.50 \times 10^8$  t of agro-residues, meaning that vast amounts of lignocellulosic biomass are accessible, although the majority of them are used for other reasons<sup>31</sup>. With indicative aims of achieving a twenty percent ethanol blend in gasoline by 2030, India declared in 2018 that it would expedite the promotion of biofuels under the National Policy on Biofuels. Oil Marketing Companies (OMCs) are marketing 10% Ethanol Blended Petrol (EBP) in accordance with BIS requirements in order to meet a target by 2022. The 10% ethanol blending objective would require about 450 crore liters of ethanol to meet the estimated 2021–2022 gasoline demand. An estimated 300 crore liters of ethanol are produced in India each year; the bulk is needed for the chemical and potable alcohol sectors, with the remainder going toward the EBP program. Although the Indian government has opened up new options for improving ethanol buying by OMCs, the 1G pathway may not be enough to attain 10% ethanol blending during 2021–2022. Consequently, it is vital to investigate alternative approaches or paths to augment the nation's ethanol output with the aim of mixing with gasoline. In order to meet the applicable BIS standards, the government has already permitted the purchase of bioethanol made from non-food raw materials other than molasses in December 2014. The availability of lignocellulosic excess biomass in India is between 12 and 16 crore tons annually. If the available biomass is used, the bioethanol yield can be increased to 2500–3000 crore liters annually, greatly reducing India's reliance on imported crude oil. In addition to manufacturing cellulosic ethanol, 2G ethanol biorefineries can also generate liquid CO<sup>2</sup> per dry ice, pellets (made from excess lignin), biogas (which can be improved to Bio CNG), and guaranteed-quality compost. The use of lignocellulosic biomass wastes can help lower greenhouse gas emissions and the pollution that comes from landfilling in the soil and water. Thus, the construction of 2G ethanol biorefineries will contribute to bettering public health. In addition to producing ethanol, lignocellulosic ethanol biorefineries have the capacity to produce high-value by-products, including xylitol, furfural, high fructose syrup, l-arabinose, etc., which could boost the bioconversion process's profitability. In this context, the Ministry of Petroleum and Natural Gas announced on August 4, 2017, the launch of the Pradhan Mantri Jaiv Indhan- Vatavaran Anukool Fasal Awashesh Nivaran (JI-VAN) Yojana, an initiative to finance 2G biorefineries<sup>32</sup>. The Ministry of Petroleum and Natural Gas has stated in 2019 that there is an initiative to develop, build, and operate ten demonstration-scale and twelve commercial-scale second generation bio-ethanol production facilities using agricultural and forestry biomass feed stocks, as well as other renewable feed stock sources.

### **CONCLUSION**

The shift toward 2G biorefineries represents a critical milestone in the pursuit of a circular bio-economy. While 1G technologies provided the initial momentum for ethanol blending, their reliance on food-grade feedstocks presents long-term sustainability challenges. 2G ethanol, derived from non-food lignocellulosic waste, offers a superior environmental profile by significantly reducing greenhouse gas emissions and utilizing surplus agricultural residues.

However, the commercialization of 2G ethanol remains contingent on overcoming high production costs and technical complexities associated with the recalcitrance of biomass. Advancements in green pretreatment solvents, such as ionic liquids, and the development of robust, co-fermenting microorganisms are essential for improving process yields. In the Indian context, the strategic implementation of 2G biorefineries is no longer a choice but a necessity to meet the 20% ethanol blending mandate (E20) and beyond. With strong government backing through interest subvention schemes and the Global Biofuel Alliance, India is well-positioned to lead the 2G revolution, provided that supply chain logistics for biomass collection are streamlined and the "waste-to-wealth" ecosystem is fully realized.

**CONFLICTS OF INTEREST:** Conflicts of interest are not present.

**ACKNOWLEDGMENTS:** The authors would like to thank Maya Devi University for its kind support during this project.

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