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REVIEW ARTICLE

BIOETHANOL, THE FUTURE FUEL, DEFINITION, HISTORY, PREPARATION AND GENERATIONS: A COMPLETE REVIEW

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ABSTRACT

The present paper is an effort to clarify the bioethanol basics and giving a scope on the bioethanol production process, and its production level worldwide. A brief Narration of the bioethanol history, with the emphasis on the types of the feedstock that were used in different literatures, and the factors that can affect the production of bioethanol. At the same time, clarify the processes of ethanol production including the pretreatment, fermentation, and distillation, and an explanation of the four different generations of the bioethanol.

KEYWORDS

renewable energy, biofuel, bioethanol, fermentation, Greenhouse gasses

1. INTRODUCTION

The world is facing a great challenge presented by the continuous population growth which subsequently rise the demand for energy sources, for the different aspects of life such transportation, heating, and industrial process (Rdhaiwi et al., 2024). These needs are meet by dependence on the fossil fuel but that rase the concerns about depletion and the different drastic change to the environment and the effect on the climate by the continuous release of greenhouse gases (GHG) (DOE (U.S. Department of Energy, 2011). That provoke the scientist to search for alternative fuel that can be sustainable and ecofriendly fuel (Mohsen and Hamza, 2023).

That lead the way for exploring the different sustainable sources of energy including solar, wind, hydrological, geothermal and biofuel energy to overcome this environmental crisis (Ang et al., 2022). Biofuel has a great potential as renewable energy, since that it can help in reducing the amount of pollutant released, as well as, the carbon oxides and other GHG released (Shivale et al., 2024). Bio fuel can be defined as any fuel (solid, liquid and gas) produced from organic biomass (Fiala and Nonini, 2018).

Biofuel can be presented in many forms such as bioethanol, biodiesel, and biogas according to the biowaste type, and how it was made (Azeez et al., 2015). Bioethanol produced by bacteria and yeast is consider as an alternative for gasoline with a higher-octane number, and it can act as octane enhancer when added to the regular gasoline (Busic et al., 2018). Bio-ethanol has the chemical formula C_2H_6O, and it had a high oxygen content which denoted it as a good alternative for fossil fuel (Alhamd et al., 2024). All the above-mentioned properties explain the growing interest in bioethanol from both environmental and economical point of view.

2. BIOETHANOL PRODUCTION HISTORY

The use of bioethanol is not a modern trend it was established in the nineteenth century, it was first used as street lamps fuel in 1850, while Nicolaus Otto was the first one to use ethanol in a four-stroke internal combustion engine in 1876 (Bertrand and Dussap, 2022). On the other hand, Hennery Ford was one of the early supporters of using ethanol, and he had designed his first T-model car to run on ethanol in 1906 (Frazier, 2008). After that the ethanol was used as fuel enhancer, but the real breakthrough in the bioethanol production was at the period from 1973 to 1979 when the fuel crises promote bioethanol production to overcome the increasing need for fuel, United States of America (UAS) and Brazil initiated a program called (Proalcool) to produce ethanol from plants with high starch and sugar content (Kazmi et al., 2025).

In 1990 the methyl tertiary butyl ether (MTBE) was banded as fuel enhancer for its influence on the environment epically the underground water, while the ethyl tertiary ether (ETBE) which was used for the first time in France in 1992, was more suitable since it meet the air pollution reduction requirement (Yee et al., 2013). In present time, ethanol production is mainly depending on starch and sugarcane and the scientists also study the use of inexpensive lignocellulosic biomass (Gong et al., 2022). The bioethanol manufacturing is getting bigger with time to fulfill the need for energy (Al-Ali et al., 2023).

The global ethanol production of bioethanol was elevated from 18.1 billion liters in 2000 to 104.7 billion liters in 2010 (Moyo et al., 2014). The bioethanol production was highly increased during the covid-19 pandemic in 2020 which was consumed as disinfectant, and the production continue to increase up to 2023, while there will be a drop in 2024 due to the economic crises (USDA (United States Department of Agriculture), 2024).

3. MAIN PRODUCERS OF BIOETHANOL WORLDWIDE

The production of bioethanol has increased significantly through time up to date, since it is considered as a great competitive for gasoline (Ayyanna et al., 2023). The main producer of bioethanol is USA followed by Brazil (Kazmi et al., 2025). The Global ethanol production was increase significantly from almost 13 billion gallons in 2007 to 30 billion gallons in 2023 with USA and Brazil as the highest producers as illustrated in Figure 1 (Ghazali and Mustafa, 2025).

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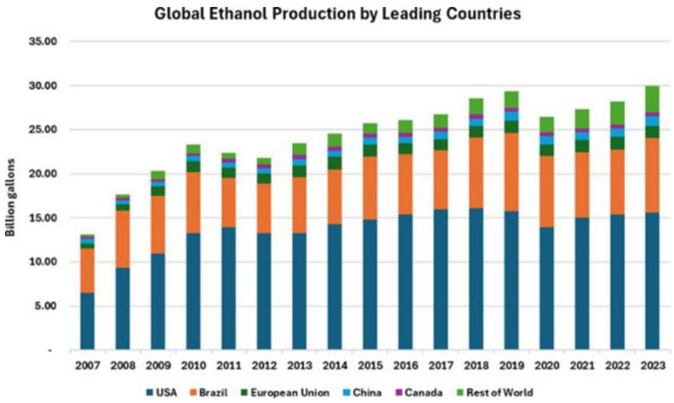

Figure 1: The global ethanol production through the period from 2007 to 2023 (Ghazali and Mustafa, 2025)

According to the renewable Fuel Association in 2024 the USA represented the largest producer worldwide with 52% of the global production, then Brazil with 28%, India with 5%, European Union with 5%, China with 4%, and the rest of the world represent only 6% of the global ethanol production as shown in Figure 2, the total ethanol production in 2024 was 31.210 billion gallons (RFA (Renewable Fuels Association) 2025).

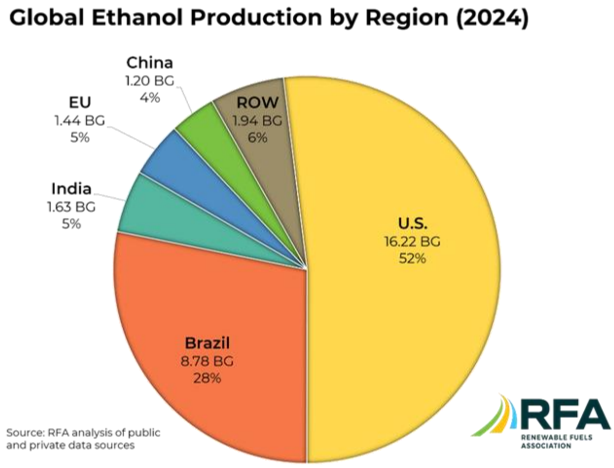

Figure 2: The global ethanol production and the amount of ethanol (Billion Gallon) the produced in 2024 (RFA (Renewable Fuels Association) 2025).

4. Properties And Advantages Of Bioethanol

Rising demand for energy resources alongside climate changes have rase the attention to this urgent subject of finding alternative energy source presented by the biofuel, and from these biofuels the bioethanol is favored due to the following characteristics (Osman et al., 2021):

- It has a noticeable smell, it is recognized as volatile as well as combustible liquid, its capability to form hydrogen bonds gave the ethanol polar properties, furthermore, it has boiling point is78.32°C.
- it has a very high production rate and due to its liquid nature its more easy to transport and store, and it release greenhouse gas emissions (12 % lower) when used in comparison to gasoline, and reduce the depending on fossil fuels which subsequently minimize variation in oil price (Fogel, 2007; Bello et al., 2020).
- 3- it has a higher-octane number, and evaporation temperature coupled with the decrease in greenhouse gases emissions. When using ethanol, with a higher oxygen content and lower hydrocarbons, NOx and other particulate matter emissions. Therefore, it is estimated that it would be a very good fuel for vehicles engine in the future, while at the same time lower air pollution (Bongartz et al., 2021; Bello et al., 2020).

5. Types Of Biomass For Bioethanol Production

A lot of countries try to rise use of bio-ethanol and lower the fossil fuels dependence in the search for energy sources that are renewable and sustainable, also mitigating environmental and protect our planet. Most of the bioethanol produced worldwide use sugar cane and corn as feedstock (Al-Ali et al., 2023).

The biomass used for bioethanol production can differ such as corn, sugarcane, potatoes, wheat straw, barley, wheat, molasses as well as date residues, the bioethanol produce from lignocellulosic materials such as agricultural residues characterized by cost-effective and ecologically benign (Mohanty and Ismail Abdullahi, 2016).

There are several factors affecting the selection of biomass that used as bioethanol feedstock which include biomass availability, cost, regional climate, as well as being ecofriendly and sustainable biomass (Shahi and Singh 2022).

Table 1: Overview of commonly reported feedstocks and microorganisms used for bioethanol production, classified according to bioethanol generation.

Feedstock	Organism	Bioethanol Generation	Reference
sugar beet	*Saccharomyces cerevisiae*	1st generation	Hinkova and Bubnik, 2000
molasses	Saccharomyces cerevisiae	1st generation	periyasamy et al., 2009
sugarcane bagasse	Saccharomyces cerevisiae	1st generation	Parveen et Al., 2023
switchgrass,	Saccharomyces cerevisiae	2nd generation	yang et Al., 2009
pine wood chips,	Saccharomyces cerevisiae	2nd generation	Cotana et Al., 2013
Gamba grass	*Zymomonas mobilis* *(Saccharomyces cerevisiae*	2nd generation	Bagudo et al., 2014
wheat straw	Saccharomyces cerevisiae	2nd generation	smuga-Kogut et Al., 2015
west Indian jasmine Rangoon creeper flowers	*S. cerevisiae*	2nd generation	Appe et al., 2018
corn stover	Saccharomyces cerevisiae. Candida CBE002	2nd generation	Astorgga-Terjo et Al., 2022
municipal waste (newspaper)	Saccharomyces cerevisiae	2nd generation	Edeh and Ezeibe, 2023
Microalgae (chlorella vulgaris)	Saccharomyces cerevisiae	3rd generation	Salman et Al., 2025
genetically modified crops (genetically modified and mutant wheat and barley straws)	Saccharomyces cerevisiae	4th generation	Li et al. 2011

5.1 Bioethanol Production Factors

Various factors that affect bioethanol production such as temperature, concentration of sugar and its type, solution pH, fermentation period, agitation speed and the size of microorganism inoculum increase in the incubation temperature may cause denaturation of the enzymes which reduce the enzyme activity (Zabed et al., 2014). The pH is also a crucial element, since it can affect the yeast growth, fermentation rate and by-product formation.

5.2 Temperature

Temperature is the most critical element that can affect the fermentation process and subsequently the bioethanol production, so it is very important to find the optimum temperature for fermentation Which can be different depend on the organism used for the fermentation (Alashmawe et al., 2018). The temperature can affect both organisms' survival as well as bioethanol production significantly (Salam et al., 2024). Enzymatic activity is regulated by the temperature and it is very sensitive to the high temperature, for optimum activities and growth an optimum temperature must be obtained (Yadav et al., 2024). A suitable temperature is essential for yeast to give an optimal fermentation as well as an optimal bioethanol production, since that very high temperature could kill the yeast while a low temperature can cause decelerate the fermentation process (Salihu et al., 2023).

5.3 Sugar Concentration and Type

The ethanol production by fermentation process depend directly on the carbohydrate (sugar) concentration and availability in the medium (Gumienna et al., 2014). The sugar concentration has a major impact on the ethanol production by yeast and finding the optimal sugar concentration is very important since that a very high sugar content could hinder the fermentation process due to substrate inhibition or accumulation of sugar residue (Gasmalla et al., 2012). The high sugar content can cause a decrease in the bioethanol production due to the increase in the osmotic pressure on the organism (Cazetta et al., 2007).

Also, the type of sugar presented in the substrate effect the amount of ethanol produce found that using a substate that only contain glucose produce an ethanol amount less than a substrate of mixture of monosaccharides and disaccharides since that the first substrate could increase the osmotic pressure and hinder the fermentation process (Emberlin et al., 2018).

5.4 Solution pH

The medium pH has an important role since that it can dictate metabolic activity of the organisms and the ethanol production as well as regulating the biomass growth (Kumar et al., 2023). The fermentation pH value can control the transition between the acidogenesis (acetic acid production) and solventogenesis (ethanol production) (Kundiyana et al., 2011). It worth mention that the solution pH effects the yeast enzyme activity since that there is an optimal pH value for each enzyme and the yeast control their intercellular pH by pump in and out H+ ions to sustain its metabolic integrity (Narendranath and Power, 2005).

5.5 Fermentation Period

The incubation period is an important factor that can affect the ethanol production significantly, the ethanol production increase as the incubation period increase since it increases the sugar conversion to alcohol (Hashem et al, 1980). While the short incubation time may affect the total ethanol production whereas short incubation period could lead to uncompleted formation of the sugar presented in the substrate (Jayus et al., 2016). On the other hand, a prolonged incubation may result in some loss of the produced ethanol due to consumption or evaporation (Caro et al., 1992). The optimum incubation period depends significantly on the organism and substrate type (Hashem et al, 1980).

5.6 Agitation Speed

The agitation speed can affect the fermentation process through its effect on the solution pH, since the increase in the agitation speed could lead to increase in the solution pH which related to the increase dissolved oxygen entry due to the high agitation (Permatasari, et al., 2023). While, the high agitation speed can cause a decrease in the glycose yield, cellulose conversion, as well as on the enzymatic cellulose hydrolysis (Solange et al., 2008). An adequate agitation can lead to good aeration, mixing, heat transfer, and enhance the the mass transfer with keeping homogeneous of the culture, but a very high agitation speed can put a shear force on the microorganisms and cause a morphological change in their cells (Muregi et al., 2021). Furthermore, it can improve the nutrients consumption by the microorganisms, and subsequently enhance their growth and tolerance (Mohammed et al., 2021).

6. Bioethanol Production

Year after year, numerous milestones and advancements have been formed to develop bioethanol production methods (Jena et al., 2023). Particularly, enhancement produced to the fermentation technology and the search and identifying many new microbial strains that can elevated the quantity and the quality of bioethanol (Nicola et al., 2011). The production of bioethanol includes important essential stages, which are pretreatment of biomass, fermentation by using different microorganisms, and finally, distillation and dehydration processes to achieve pure bioethanol (Shahi and Singh, 2023.). The pretreatments process for lignocellulosic feedstock is crucial step which allow the cellulose to be freed from the matrix of polymers consisting of lignin and hemicellulose in which it naturally existed. It this case the cellulose will become more accessible for hydrolysis by microorganism enzyme since that its crystalline structure is disrupted, which lead to increased sugar yields that using as a feedstock.

Without the pretreatment the hydrolyzing enzyme can't reach the cellulose instead it will be attached to the lignin surface and ultimately hinder the fermentation process (Ceballos, 2018). There are a variety of pretreatment methods and choosing the best pretreatment method depending on many factors such as the biomass type (woody or non-woody) which differ in their matrix complexity. Woody biomass including both hardwoods and softwoods, contain a higher lignin content which make them harder to process. Such as eucalyptus and pine wood, which are utilized for industrial bioconversion application. while, biomass consist of agricultural residues such as wheat straw, corn cobs, sugarcane bagasse, corn husks, soybean hulls, and grasses are considered as non-woody biomass (Mankar et al., 2011).

The pretreatment methods can be classified to either traditional or advanced pretreatments methods. Traditional pretreatments can be divided as four main types which are chemical, physical, physicochemical, and biological methods whereas advanced pretreatment techniques can be narrow down to acid-based or ionic liquid-based fractionation, respectively (Singla and Negi 2015). Chemical pretreatments is efficient in dissolving, hydrolyzing, or oxidizing the biomass, which allow their conversion, but it may cause the production of by-products that are toxic to the microorganisms the chemical methods can ranged from acid, alkali, oxidative delignification, to organic acid (organo-solvent) methods, these methods are selective which mean it hydrolysis a specific component of the feedstock, and in most cases the target component is lignin and/or hemicellulose, but in the same time the using of these pretreatment have a disadvantage represented by difficult operating and the generation of by-products (Harmsen et al., 2010; Brodeur et al., 2011).

Physical pretreatments are efficient in disturbing lignocellulosic biomass, enabling their conversion into simpler sugars that can be utilized for the bioethanol production, this involves the breaking down lignocellulosic biomass size and crystallinity through the use of physical techniques including milling, grinding, extrusion and irradiation (ultrasound, microwave, gamma rays, and electron beam) (Sant'Ana Júnior et al., 2025). In general, the physical pretreatments have the ability to decrease the particle size while increasing the particle surface area and disturbing the lignocellulosic structure which enabling increase in the enzymatic hydrolysis efficiency. no inhibitor products are present in these methods and they are faster, but they need specific equipment and/ or conditions to get the required results (Kumar et al., 2008). Whereas, fermentation involves the conversion of the monomeric units of sugars to ethanol, through the fermentation activities of microorganisms such as yeast, fungi or bacteria (Niphadkar et al., 2018).

In fermentation the microbial activities alter feedstock material to produce fermentation products. The parameters that effect the fermentation include microbial inoculum, concentration of the reducing sugar, the medium pH, and incubation temperature increasing the fermentation period will lead to rise the bioethanol concentration; but after reaching the optimal fermentation time, there will be a decrease in the ethanol concentration when extending the fermentation period (Febriani et al., 2025). The fermentation time or period is related to the development stage of microbes during the fermentation, which subsequently affects the fermentation yield. Distillation process, a process in which a mixture of liquid or vapor was separated to obtain pure fractions. in ethanol distillation the fermentation solution was heated and the ethanol vapor in distillation column was condensation and cooled to converts alcohol vapor into liquid, which result in separating ethanol from the mixture then it was storage until their transportation to market (Adi et al., 20202; Nassif et al., 2022). The main parameters that need to be monitor during distillation are the rate of distillation, concentration of ethanol in the distillate, the yield. resulted from distillation process, as well as temperature (Febriani et al., 2025).

7. Bioethanol Generations

As bioethanol technology has evolved, it has been categorized into four generations, each representing distinct innovations aimed at enhancing sustainability and efficiency in biofuel production. However, the researchers divided bioethanol produced into four main types depending on feedstock type. The first generation resulted from the fermentation of edible food crops; while, the second generation resulted from the fermentation of non-edible crops and waste materials; on the other hand, the third generation resulted from the fermentation of different algae and microbes, the fourth generation resulted from the genetically modified microbes (Cavelius et al., 2023).

7.1 First Generation Bioethanol

1st generation bioethanol use food crops rich in starch and sugar as feedstocks for the production of bioethanol such as potato, corn and sugarcane that required a hydrolysis process for breaking down complex carbohydrates (Guimarães et al., 2023; de Mello et al., 2022). Despite the environmental benefits of first-generation biofuels, concerns have been arisen for the following reasons regarding their large-scale production. which include the competition for essential resources such as water and arable land, potentially driving up food commodity prices and may lead to food scarcity. However, it is important to note that 1st generation biofuels lower levels of GHG emissions when compared to gasoline.

7.2 Second Generation Bioethanol

The primary source of 2nd generation of bioethanol produced from lignocellulosic biomass, although industrial waste products like whey or crude glycerol can also be utilized as raw materials. Lignocellulosic biomass characterized by it is locally abundant and cost-effective, but the converting of lignocellulose into reducing sugars is complex and required pretreatments. Lignocellulosic bioethanol offers a promising and environmentally sustainable alternative due to its lower emission of greenhouse gases and reduced air pollution compared to first generation bioethanol (Abd Alsaheb, et al., 2025).

7.3 Third-Generation Bioethanol

The 3rd generation biofuels arise as a solution for the problematic situation presented in the first and second generations biofuel including the competition with food for land and resources. Therefore, researchers and scientists focused on finding solution for this problem, however, it requires specific techniques that are costly and not acceptable from economic point of view (Alam et al., 2015; Brennan and Owende, 2010). The 3rd generation bioethanol utilizes algae to produce bioethanol, that fixes the problems of the past two generations since it has high biomass productivity and dose not competition with the lands required for food production. But it is limited by the difficulty of large-scale algae production and energy-intensive processing technology. Microalgae species such as *Botryococcus, Chlorella*, and *Nannochloropsis* capture CO2 from fossil fuel combustion, thus contributing to global climate change mitigation (Ferreira et al., 2021). Although oils extracted from microalgae have the potential to replace conventional vegetable oils in biodiesel production, the technology for efficient oil extraction still requires further development.

7.4 Fourth-Generation Bioethanol

Metabolic engineering of microorganisms for biofuel production forms the basis for fourth-generation biofuel production which can meet the need for lowering biofuel costs, greenhouse gas emissions, and land and water resource needs (Moravvej, et al., 2019). The fourth generation depend mainly on the fourth generation of bioethanol production involves advanced technologies such as genetic engineering and synthetic biology to create microorganisms capable of directly converting CO2 into bioethanol or improving the efficiency of bioethanol production. The goal of fourth-generation bioethanol is to be carbon neutral or even carbon negative, capturing more CO2 during production than is emitted over the entire lifecycle of the bioethanol (Abid et al., 2022). 4th generation biofuels use a nonarable land and the application of both genetically modified feedstock and gnomically altered microorganisms to obtain bioenergy. Scientists where able to modify the genetics and metabolic pathways of microorganisms to rise the metabolites production (Nassif et al., 2022).

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