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Comprehensive assessment of Bioethanol: An Integrated Analysis of Production Processes, Utilization, Advancement and Environmental Implications

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Abstract: Bioethanol is examined in the abstract as a potential alternative with its own set of technical and quality considerations, and it highlights the urgency of moving away from fossil fuels. The production of Bioethanol reduces the dependency on imported fossil fuels, reduces the emission of exhaust gases when it is used as alternative energy source. Bioethanol has been identified as the mostly used biofuel worldwide since it significantly contributes to the reduction of crude oil consumption and environmental pollution. It can be produced from various types of feedstocks such as sucrose, starch, lignocellulosic and algal biomass through fermentation process by microorganisms. Compared to other types of microoganisms, yeasts especially Saccharomyces cerevisiae is the common microbes employed in ethanol production due to its high ethanol productivity, high ethanol tolerance and ability of fermenting wide range of sugars. However, there are some challenges in yeast fermentation which inhibit ethanol production such as high temperature, high ethanol concentration and the ability to ferment pentose sugars.Bioethanol is one of the most interesting biofuels due to its positive impact on the environment. Currently, it is mostly produced from sugar- and starch-containing raw materials. However, various available types of lignocellulosic biomass such as agricultural and forestry residues, and herbaceous energy crops could serve as feedstocks for the production of bioethanol, energy, heat and value-added chemicals. Lignocellulose is a complex mixture of carbohydrates that needs an efficient pretreatment to make accessible pathways to enzymes for the production of fermentable sugars, which after hydrolysis are fermented into ethanol. For obtaining high yield of Bioethanol it requires advancement in production process and genetic modification in strain through genetic engineering and metabolic engineering.

Keywords: Bioethanol; Biofuels; Alternative energy source; Biomass(Lignocellulosic Biomass), Advancement; Generation; challenges.

I. INTRODUCTION

Continuous depletion of fossils energy reserves, greenhouse gas emissions (GHG), energy security, raised demand for fuels and energy and economic development are critical concerns in today's scenario[1]. On a global basis, the transportation sector has increased CO2 emissions by 24% (8.2 Gt in 2019) and is expected to grow to 1.3 billion vehicles by 2030 and 2 billion by 2050 (IEA, 2020). As a result, the search for renewable alternatives for the promotion of renewable fuels, energy, and chemicals has been researched in recent years (Chandrasekhar et al., 2021a, Chandrasekhar et al., 2021c)[2]. In view of this advanced bioenergy has displaced substantial contribution to meet the current energy demand accounting for one-tenth of the world's total primary energy demand (https://www.iea.org/fuels-and-technologies/bioenergy)[3]. According to the Renewable 2019 published report, worldwide biofuel output climbed by 10% in 2018 to 154 billion liters, and is expected to expand by 25% by 2024 (Raturi, 2019). Transportation biofuels production has been extended to 6% in 2019 and is predicted tto grow at a rate of 3% per year over the next five years, reaching 10% by 2030 . [4]. Bioethanol, a renewable and environmentally friendly alternative to fossil fuels, has gained significant attention worldwide as a viable solution to mitigate climate change and reduce dependence on finite energy resources[5]. Concerning the continuously increasing global demand for energy, fossil fuel resources on our planet are anticipated to become depleted within the next several decades, endangering worldwide energy security[6]. More importantly, the combustion of fossil fuels contributes to CO2 emissions and hence global warming, a rise in sea levels, urban pollution, and loss of biodiversity, constituting a threat to the global environment. Therefore, the energy transition to lowcarbon-intensity fuels becomes necessary to tackle climate change[7]. Produced primarily through the fermentation of biomass, such as corn, sugarcane,starch-

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containing raw materials, soybeans, wheat, and lignocellulosic materials, bioethanol offers several advantages, including lower greenhouse gas emissions, improved energy security, and rural development opportunities[8]. Bioethanol is an alternative fuel and the production process typically involves adding substrates, culture medium, and nutrients into a fermentor containing active microorganisms, such as yeast, and withdrawing the products[9]. Bioethanol is a high octane number fuel and has a positive impact on the environment[10]. It also addresses the growing importance of bioethanol as a sustainable energy source in the face of increasing concerns about climate change and energy security[11]. Bioethanol account for 65% of total biofuels and play a critical role in ensuring national energy and economic security (IEA, 2020). Although, advanced biofuels contribute 5.0% to total energy consumption and are continuously improving their environmental benefits, cutting GHG by 39–46% in the case of corn ethanol. Bioethanol is an advanced clean liquid biofuel[12]. It aims to explore various aspects of bioethanol production at an international level, from feedstock selection to environmental considerations and future directions[13]. The global pursuit of sustainable energy solutions has propelled bioethanol production into the spotlight as a promising avenue for reducing greenhouse gas emissions and fostering energy independence[14]. As countries worldwide seek to transition towards cleaner energy sources, bioethanol has emerged as a key player in the renewable energy landscape[15]. It underscores the significance of bioethanol as a renewable alternative to fossil fuels and highlights the collaborative efforts among nations to advance research, innovation, and implementation in this field[16].

II. DEFINITION OF BIOETHANOL

Bioethanol is ethanol (C2H5OH), or ethyl alcohol, produced by biological methods[17]. It is a renewable, non-toxic, and biodegradable resource that helps reduce greenhouse gas emissions and our dependence on crude oil[18]. Bioethanol can be blended with conventional fuel without the need for engine modifications, and in quantities up to 5%, it can be blended with gasoline[19]. Fuel vehicles can run on up to 85% ethanol and 15% petrol blends (E85)[20]. Bioethanol is also used to heat rooms, as is the case with bioethanol fireplaces and heaters. Bioethanol is divided into three types, depending on the raw material used for its production[21]. Bioethanol is alcohol produced by the fermentation of starch and lignocellulosic substrates via microorganisms, releasing recyclable carbon dioxide, water and heat[22]. The carbon dioxide released by the combustion of bioethanol can recycle in microalgae production as a carbon source[23].

III. IMPORTANCE OF BIOETHANOL: A COMPREHENSIVE ANALYSIS

Bioethanol, a renewable fuel derived from organic matter such as crops, agricultural residues, and organic waste, plays a pivotal role in addressing pressing global challenges related to energy security, climate change mitigation, economic development, and environmental sustainability[24]. In this comprehensive analysis, we delve into the multifaceted importance of bioethanol across various domains[25]. **Renewable Energy Source:** Bioethanol offers a sustainable alternative to finite fossil fuels by utilizing biomass resources that can be continually replenished through agricultural practices. Unlike fossil fuels, which are nonrenewable and contribute to environmental degradation and climate change, bioethanol production harnesses the power of photosynthesis to convert solar energy into liquid fuel[26]. **Carbon Reduction**: When used as a fuel, bioethanol emits fewer greenhouse gases compared to conventional gasoline. The carbon dioxide released during combustion is offset by the carbon absorbed by the plants during their growth phase, resulting in a closed carbon cycle. As a result, bioethanol helps mitigate climate change by reducing overall carbon emissions and decreasing reliance on carbonintensive fossil fuels[27]. **Energy Security:** Bioethanol production enhances energy security by reducing dependence on imported fossil fuels. By utilizing domestically available biomass resources, countries can mitigate the risks associated with volatile oil markets, geopolitical tensions, and supply disruptions. This localized approach to energy production promotes national resilience and autonomy[28]. **Economic Development**: The bioethanol industry stimulates economic growth by creating jobs in agriculture, processing, distribution, and related sectors. Farmers benefit from additional revenue streams through the cultivation of energy crops, while rural communities experience increased investment and infrastructure development. Moreover, the growth of the bioethanol market attracts private sector investment and fosters innovation in renewable energy technologies[29]. **Diversification of Energy Sources:** By diversifying the energy mix, bioethanol reduces reliance on a single energy source, enhancing energy resilience and stability. This diversification not only mitigates the risks associated with fluctuations in oil prices but also promotes technological innovation and competition within the energy sector[30]. **Agricultural Revenue**: Bioethanol production provides an additional market for agricultural commodities, increasing demand for crops such as corn, sugarcane, and dedicated energy crops. This creates new revenue opportunities for farmers and incentivizes sustainable agricultural practices. Additionally, bioethanol production utilizes agricultural residues and organic waste, further enhancing resource efficiency and waste management[31]. **Advanced Technologies:** Ongoing research and development in

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bioethanol production technologies drive innovation across multiple disciplines, including agriculture, biotechnology, chemistry, and engineering. Advanced biofuel processes, such as cellulosic ethanol production and biochemical conversion pathways, offer greater efficiency, scalability, and environmental sustainability compared to traditional fermentation methods[32]. **Flex-Fuel Vehicles:** FBioethanol can be blended with gasoline in various proportions, enabling the use of flex-fuel vehicles that can run on both bioethanol and gasoline. This flexibility provides consumers with more fuel choices, reduces dependence on petroleum, and promotes the widespread adoption of bioethanol as a renewable transportation fuel[31]. **Reduced Fossil Fuel Consumption**: Increased use of bioethanol displaces fossil fuel consumption in the transportation sector, reducing overall greenhouse gas emissions and fossil fuel depletion. As governments implement renewable fuel standards and carbon pricing mechanisms, the demand for bioethanol continues to grow, driving further investment in renewable energy infrastructure[32]. **Air Quality Improvement:** Bioethanol use in vehicles leads to lower emissions of harmful pollutants such as sulfur dioxide, nitrogen oxides, and particulate matter. This contributes to improved air quality, public health, and environmental conservation, particularly in urban areas with high levels of vehicular emissions[33]. **Waste Utilization**: Bioethanol production can utilize organic waste streams such as agricultural residues, food waste, and forestry residues, providing an environmentally friendly solution for waste management. By converting waste into valuable biofuel feedstocks, bioethanol production reduces landfill waste, greenhouse gas emissions, and environmental pollution[34]. **Policy Support**: Government policies play a critical role in promoting bioethanol production and consumption through renewable fuel standards, tax incentives, subsidies, and mandates. These policy measures create market incentives for investment in biofuel infrastructure, research, and development, driving innovation and market growth in the bioethanol industry[35]. **International Cooperation:** Bioethanol production fosters international cooperation and trade partnerships, with countries exchanging knowledge, technology, and biofuel resources to meet global energy and climate goals. Collaborative initiatives such as joint research projects, technology transfer agreements, and trade agreements promote the sustainable development of bioethanol resources on a global scale[36]. **Energy Independence:** Domestic bioethanol production reduces reliance on imported oil and enhances energy independence, mitigating geopolitical risks associated with oil dependence and supply disruptions. By harnessing locally available biomass resources, countries can strengthen their energy security and reduce vulnerability to global oil market fluctuations[37]. **Carbon Sequestration:** Energy crops used for bioethanol production sequester carbon dioxide from the atmosphere during photosynthesis, offsetting emissions from bioethanol combustion and contributing to carbon sequestration. This carbon-neutral or even carbonnegative aspect of bioethanol production enhances its environmental sustainability and climate change mitigation potential[38]. **Adaptation to Climate Change**: Bioethanol offers a sustainable energy solution in the face of climate change, providing a resilient alternative to fossil fuels vulnerable to supply chain disruptions and extreme weather events. As countries strive to reduce their carbon footprint and transition to low-carbon energy systems, bioethanol plays a crucial role in building climate resilience and mitigating the impacts of climate change on vulnerable communities[39]. **Community Engagement:** Bioethanol projects often involve local communities in decisionmaking processes, promoting community engagement, social responsibility, and environmental stewardship. By fostering partnerships with local stakeholders, bioethanol producers can address community concerns, ensure equitable distribution of benefits, and enhance the social acceptance of bioenergy projects[40]. **Education and Awareness**: Bioethanol production and utilization raise public awareness about renewable energy, sustainability, and environmental conservation, fostering a culture of responsible energy consumption and stewardship. Through educational programs, outreach initiatives, and public engagement efforts, stakeholders can empower individuals to make informed choices and support the transition to a more sustainable energy future[41]. **Infrastructure Development:** Investment in bioethanol infrastructure, such as refineries, distribution networks, and fueling stations, creates jobs and supports economic growth in both urban and rural areas. As bioethanol production scales up to meet growing demand, there is a need for robust infrastructure investments to ensure efficient production, transportation, and distribution of biofuels[42]. **Technological Advancements:** Advances in bioethanol production technologies improve efficiency, reduce costs, and expand the range of biomass feedstocks, making bioethanol more competitive and accessible as a renewable energy source. From genetic engineering and biomass pretreatment to fermentation optimization and process integration, ongoing research and innovation drive continuous improvements in bioethanol production processes and systems. bioethanol plays a critical role in addressing the complex challenges of energy security, climate change mitigation, economic development, and environmental sustainability[43]. As governments, industries, and communities worldwide embrace renewable energy solutions, bioethanol emerges as a key driver of the transition to a more sustainable and resilient energy future[44].

IV. IMPACT ON FOSSIL FULES FROM BIOEHTHANOL PRODUCTION

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The production of bioethanol has both positive and negative impacts

Reduced greenhouse gas (GHG) emissions: Bioethanol production can result in lower GHG emissions compared to fossil fuels, as it is derived from renewable sources[45]. **Increased GHG emissions**: However, changes in land use patterns for bioethanol production can lead to increased GHG emissions, especially if deforestation or biodiversity loss occurs[46]. **Reduced fossil fuel imports:** Bioethanol production can lead to lower fossil fuel imports, reducing dependence on foreign oil and potentially increasing energy security and gives high performance as compared to fossil fuels[47]. **Economic benefits:** Bioethanol production can create jobs and stimulate economic growth, especially in rural areas where feedstock is grown[48]. **Environmental concerns:** Bioethanol production can have negative environmental impacts, such as soil erosion, nutrient depletion, and water pollution[49]. **Air quality:** Bioethanol combustion generally produces fewer emissions of particulates, sulfur dioxide, and other pollutants compared to fossil fuels[50]. **Water usage**: Bioethanol production can require significant amounts of water, which can put pressure on water resources in some regions[51]. **Food prices:** The production of bioethanol can sometimes lead to increased food prices due to competition for land and resources[52]. **Technological challenges**: The production of bioethanol can be technologically complex, with challenges in achieving costefficient upscaling and addressing environmental concerns[53]. **Government policies:** Government programs and regulations, such as the U.S. Renewable Fuel Standard (RFS) and California's Low Carbon Fuel Standard (LCFS), can influence the production and use of bioethanol and its impact on fossil fuels[54].

V. BIOETHANOL ACT AS A ALTERNATIVE ENERGY SOURCE

Bioethanol is considered a renewable energy source that provides a sustainable environment by reducing carbon dioxide emissions with an impact on climate change. Bioethanol is produced by yeast fermentation from different feedstocks, and it is a high octane number fuel that can be used as an alternative to fossil fuels[55]. Bioethanol has several advantages, including reducing dependence on fossil fuels, which can be replaced with fuels from renewable plant sources. Bioethanol is also biodegradable, making it a more environmentally friendly fuel option compared to fossil fuels[56]. However, the biggest challenge remains how to reduce the production cost of bioethanol. The biorefinery concept is needed to utilize renewable feedstocks more comprehensively and to produce bio-based materials that would reduce the cost of bioethanol production[57]. Bioethanol has been shown to have a high octane number and can be blended with gasoline to reduce greenhouse gas emissions and limit the use of fossil fuels. Bioethanol can be mixed with gasoline in any proportion. - Bioethanol used as a fuel has a higher octane number than gasoline, with an effect on reducing fuel consumption and increasing electricity. - Ethanol combustion results in low CO2 emissions. - Compared to fossil fuels, bioethanol is biodegradable. While bioethanol can be mixed with gasoline in various proportions, there are practical limitations and considerations such as engine compatibility and regulatory guidelines[58]. Bioethanol's higher octane number compared to gasoline can indeed lead to improved engine performance, potentially reducing fuel consumption and increasing power output. Ethanol combustion generally results in lower CO2 emissions compared to gasoline, primarily due to its renewable source and the CO2 absorption during plant growth. However, lifecycle analysis is crucial to fully understand emissions impacts[59]. Compared to fossil fuels, bioethanol is indeed biodegradable, making it less harmful to the environment in case of spills or leaks. This biodegradability is one of the advantages of bioethanol in terms of environmental impact and sustainability[60].

VI. GLOBAL STATUS AND TRENDS IN BIOETHANOL PRODUCTION

The global bioethanol market is experiencing significant growth, with a value of USD 33.61 billion in 2021 and an expected CAGR of 14.1% during the forecast period, projected to reach USD 114.7 billion by 2028 .The global production of bioethanol has increased from 17.25 billion liters in 2000 to over 46 billion liters in 2007[61]. The rising demand for bioethanol in the pharmaceutical industry is due to its effectiveness as a disinfectant, its use in extraction and purification processes, its application in pharmaceutical R&D, and its compliance with regulatory standards However, the expansion of bioethanol feedstock cultivation can lead to adverse environmental impacts, such as deforestation and loss of biodiversity[62]. The growth of the bioethanol market is driven by factors such as supportive government policies, advancements in biofuel technologies, and the global push towards decarbonization the global bioethanol market is witnessing substantial growth due to increasing environmental concerns, government support, and advancements in biofuel technologies[63]. However, the industry also faces challenges related to its environmental impact. The market is projected to continue its expansion, driven by the demand for cleaner and more sustainable energy sources. **Rising Demand for Renewable Energy:** With increasing awareness of climate change and the need to reduce reliance on fossil fuels, there has been a surge in demand for renewable energy sources like bioethanol. Governments worldwide are implementing mandates and incentives to promote the use of biofuels in transportation and other sectors, driving market growth[64]. **Technological Innovations:** Advances in biotechnology and bioengineering have led to improved processes for

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bioethanol production. Novel enzymes, fermentation techniques, and genetic modifications of feedstock crops have enhanced yields and lowered production costs, making bioethanol more competitive with traditional fossil fuels[65]. **Diverse Feedstock Sources:** Bioethanol can be produced from various feedstock sources, including corn, sugarcane, wheat, barley, and lignocellulosic biomass such as agricultural residues and energy crops. This diversity in feedstock options provides flexibility to producers, allowing them to adapt to regional agricultural conditions and market dynamics[66]. **Expansion of Production Capacities**: Countries like the United States, Brazil, China, and the European Union have invested significantly in expanding bioethanol production capacities. Large-scale biorefineries equipped with advanced technologies are being established to meet growing demand both domestically and for export markets[67]. **Integration with Traditional Agriculture:** Bioethanol production has become integrated into traditional agriculture systems, providing farmers with additional revenue streams and opportunities for crop diversification. By utilizing surplus agricultural feedstocks, bioethanol production helps in reducing food waste and stabilizing commodity prices[68]. **Environmental Benefits:** Compared to conventional gasoline, bioethanol offers several environmental benefits, including lower carbon emissions and reduced air pollutants. Its use contributes to mitigating climate change and improving air quality, aligning with global sustainability goals and emissions reduction targets[69]. **Policy Support and Regulatory Frameworks:** Many countries have implemented biofuel mandates, blending targets, and tax incentives to promote the use of bioethanol. These policy measures create a favorable market environment for biofuel producers, stimulating investment in the sector and driving technological innovation[70]. **International Trade Dynamics:** Bioethanol tr ent years, driven by differences in feedstock availability, production costs, and policy frameworks among countries. Export-oriented producers like Brazil and the United States supply bioethanol to regions with limited domestic production capacity, enhancing energy security and promoting economic cooperation[71]. **Challenges in Feedstock Availability**: Despite the abundance of potential feedstock sources, competition for land, water, and resources poses challenges to sustainable bioethanol production. Balancing the need for food security with biofuel production requires careful planning and implementation of landuse policies to avoid negative social and environmental impacts[72]. **Economic Viability and Market Dynamics**: The economic viability of bioethanol production depends on various factors, including feedstock prices, production costs, energy market dynamics, and government subsidies. Fluctuations in commodity prices and energy markets can affect the profitability of bioethanol production and investment decisions in the sector[73]. **Techno-Economic Challenges:** While technological innovations have improved the efficiency of bioethanol production, challenges remain in scaling up new processes and integrating them into existing infrastructure. Cost-effective production of advanced biofuels from non-food biomass requires further research and development to overcome technical and economic barriers[74]. **Environmental Concerns and Sustainability:** Despite its environmental benefits, bioethanol production can raise concerns related to land use change, biodiversity loss, and water scarcity, especially when sourced from food crops or cultivated on marginal lands. Sustainable production practices, certification schemes, and environmental regulations are essential to ensure the long-term sustainability of biofuel supply chains[75]. **Emerging Trends in Advanced Bioethanol:** Research efforts are focused on developing advanced bioethanol technologies that utilize non-food feedstocks such as algae, waste biomass, and municipal solid waste. These next-generation biofuels offer potential advantages in terms of feedstock availability, land use efficiency, and carbon intensity reduction[76]. **Integration with Renewable Energy Systems:** Bioethanol production is increasingly integrated with other renewable energy systems such as wind and solar power to create synergies and enhance overall energy efficiency. Co-locating biofuel facilities with biomass power plants or biogas digesters can improve resource utilization and reduce environmental impacts[77]. **Global Collaboration and Knowledge Sharing:** Collaboration among governments, industry stakeholders, and research institutions is crucial for addressing common challenges and accelerating the transition to a sustainable bioeconomy. Knowledge sharing platforms, international partnerships, and collaborative research initiatives facilitate the exchange of best practices and innovation in bioethanol production[78].

VII. GENERATION OF BIOETHANOL

Bioethanol can be produced from various types of feedstocks. The first-generation bioethanol feedstock is mainly edible food crops such as rice, wheat, barley, potato, corn, sugarcane, and vegetable oil, for example, soybean. Second-generation bioethanol feedstocks are non-food crops such as switchgrass, miscanthus, and woody biomass[79]. Third-generation bioethanol feedstocks are algae and other microorganisms. Fourthgeneration bioethanol feedstocks are waste materials such as municipal solid waste, food waste, and agricultural residues. The choice of feedstock depends on factors such as availability, cost,and Sustainability[80]

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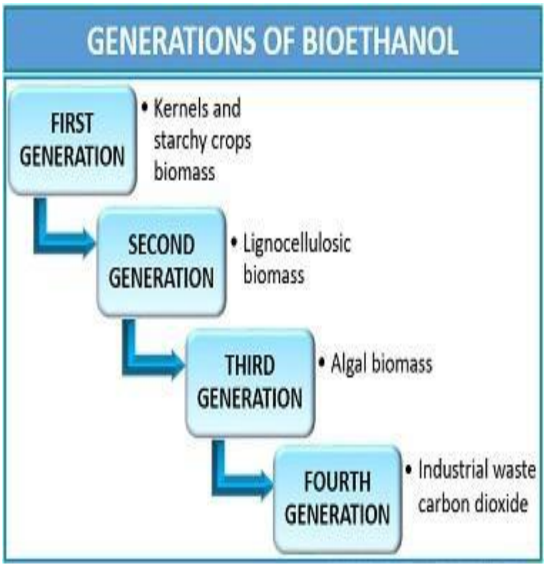

Figure: 1:Generations of bioethanol

1. **First-generation bioethanol**: It makes the use of kernels and starchy crops biomass like sugar-beet, sugar-cane, wheat, corn etc. The production process requires more land area for the cultivation of crops, due to which the capital cost in the first generation is quite higher. The bioethanol produced from such feedstocks contains a high sugar concentration comparative to the other feedstocks.
2. **Second-generation bioethanol:** It makes the use of lignocellulosic biomass like wood, straw, grass and wastes etc. The production process does not require much capital cost for the maintenance and operation of sophisticated equipment. The feedstock necessary for its production can be grown in poor quality marginal land, which produces low greenhouse gas emissions. Its conversion efficiency is low.
3. **Third generation bioethanol:** It makes the use of algal biomass whose cultivation is easy (can be cultivated on marginal land). Bioethanol derived through the cultivation of algal biomass needs low capital and has high energy density and conversion energy.
4. **Fourth-generation bioethanol:** It makes the use of industrial waste carbon dioxide. The bioethanol produced by this process is considered a carbon-negative biofuel. Further research is still going on.

VIII. Feedstock Selection And Availability

In bioethanol production, feedstock used as a raw material as a source of sugars or starches that can be converted into ethanol through fermentation. This can include various types of organic materials such as grains (corn, wheat), sugarcane, sugar beets, cellulosic biomass (agricultural residues, forestry residues, energy crops), and even algae[81]. Global estimation of the annual production of biomass production is 170 billion metric tons/ year and the international energy agency (IEA) estimates that 10% of forestry and agricultural residues contribute to 233 billion liters of bioethanol (Su et al., 2020). US Department of Energy reported producing 1.3 billion tons of LCB every year, contributing 933 million tones/ year of agricultural residue and 369 million tons/ year of forest residues (De Bhowmick et al., 2018). Whereas,in India the current availability of biomass in India is estimated at about 750 MMT per annum and surplus biomass availability at about 230 MMT per annum. The installed capacity for biomass production in India has grown at a CAGR of 4 per cent reaching 10 GW in FY2.[82]. The choice of feedstock depends on factors such as availability, cost, regional suitability, technological compatibility, and environmental impact.

Feedstock Types:

Starch-based: Corn, wheat, barley, and cassava are common sources. These contain high levels of starch, which can be converted into sugars for fermentation.

Sugar-based: Sugarcane, sugar beets, and sweet sorghum are rich in sucrose, readily fermentable into ethanol.

Cellulosic feedstock: Biomass such as agricultural residues (corn stover, wheat straw), forestry residues, energy crops (switchgrass, miscanthus), and municipal solid waste contain cellulose and lignocellulose, which can be broken down into sugars and then fermented.[83].

Availability:

Regional Considerations: Availability varies geographically based on climate, soil type, and agricultural practices. For instance, sugarcane is prevalent in tropical regions, while corn dominates in temperate climates.

Seasonality: Seasonal availability impacts feedstock choice. Some crops are harvested once a year, while others, like sugarcane, may have multiple harvests.

Yield and Growth Rate: Fast-growing crops or those with high yields per hectare are preferred to maximize output.[84]

Economic Factors:

Cost of Feedstock: The cost of acquiring feedstock significantly affects production economics. It includes cultivation, harvesting, transportation, and processing costs.

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Market Price Stability: Volatility in commodity markets can impact feedstock prices, affecting production profitability.

Co-product Value: Some feedstocks offer valuable co-products (e.g., animal feed from corn or bagasse from sugarcane), which can offset production costs.[85]. **Technological Considerations:**

Conversion Efficiency: Feedstock should be easily convertible into fermentable sugars through enzymatic hydrolysis (for cellulosic biomass) or simple extraction processes (for starch and sugar-based feedstocks).

Compatibility with Production Processes: Feedstock properties must align with the chosen production technology (e.g., starchbased feedstocks for conventional fermentation or cellulosic feedstocks for advanced biofuel processes).[86].

Environmental Impact:

Land Use: Sustainable feedstock selection considers land-use efficiency, minimizing competition with food crops and preserving natural habitats.

Water and Energy Requirements: Some crops demand intensive water or energy inputs for cultivation and processing, impacting overall environmental footprint.[89].

IX. PRETREATMENT TECHNIQUES OF BIOMASS

To treating the biomass for Bioethanol production it involves many techniques depends upon the biomass used, which are given below1.

Mechanical Pretreatment:

Milling: Biomass is physically broken down into smaller particles using mechanical forces, such as hammer mills or ball mills. This increases the surface area for subsequent chemical or enzymatic treatments.

Chipping: Involves cutting or chipping biomass into smaller pieces, typically used for larger woody biomass feedstocks like forestry substances.[90]

Chemical Pretreatment:

Acid Pretreatment: Biomass is treated with dilute acids (e.g., sulfuric acid, hydrochloric acid) under controlled conditions of temperature and pressure. This breaks down hemicellulose into monomeric sugars and partially removes lignin, making cellulose more accessible.

Alkaline Pretreatment: Involves treating biomass with alkaline solutions (e.g., sodium hydroxide, ammonium hydroxide) to disrupt lignin structure and remove hemicellulose, leading to increased cellulose accessibility.

Organosolv Pretreatment: Biomass is treated with organic solvents (e.g., ethanol, methanol) at high temperatures to dissolve lignin and break down hemicellulose, resulting in a more digestible cellulose fraction.[91] **Thermochemical Pretreatment:**

Steam Explosion: Biomass is exposed to high-pressure steam followed by a rapid decompression, causing the lignocellulosic matrix to swell and rupture, thereby increasing accessibility of cellulose to enzymatic hydrolysis.

Pyrolysis: Involves heating biomass in the absence of oxygen to break down complex organic molecules into simpler compounds, including bio-oil, biochar, and syngas.

Torrefaction: Biomass is heated at moderate temperatures in the absence of oxygen to remove moisture and volatile components, resulting in a more stable and energy-dense material. [92].

Biological Pretreatment:

White Rot Fungi: Certain fungi, such as Phanerochaete chrysosporium or Trametes versicolor, produce enzymes capable of degrading lignin, thereby increasing accessibility of cellulose to enzymatic hydrolysis.

Cellulolytic Enzymes: Enzymes like cellulases, hemicellulases, and ligninases can be applied directly to biomass to break down cellulose, hemicellulose, and lignin into fermentable sugars. [93].

Combined Pretreatment:

Sequential or Simultaneous: Different pretreatment methods can be used sequentially or simultaneously to optimize biomass breakdown and sugar release. For example, a combination of dilute acid and steam explosion pretreatment has been shown to improve ethanol yields from lignocellulosic feedstocks. Each pretreatment method have own characteristics and limitations, and depends on factors such as biomass feedstock composition, desired ethanol yield, process economics, and environmental considerations.[94].

X. PRODUCTION PROCESS:

Concerning the continuously increasing global demand for energy, fossil fuel resources on our planet are anticipated to become depleted within the next several decades, endangering worldwide energy security. More importantly, the combustion of fossil fuels contributes to CO_2 emissions and hence global warming, a rise in sea levels, urban pollution, and loss of biodiversity, constituting a threat to the global environment. Therefore, the energy transition to low-carbon-intensity fuels becomes necessary to tackle climate change. All these negative

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environmental, social, political, and energy security concerns of the current world has boosted interest in alternative energy sources, including biofuels. However, although alternative energy sources hold the key to solving the three critical global problems, i.e., energy demand and security and climate change (Figure 2), the transition from fossil fuels to more sustainable energy resources require a high initial investment and innovative technologies. Therefore, employing an energy mix of fossil fuels, biofuels, and renewable energy sources seems to be a good starting strategy to switch to solely sustainable resources in the near future.

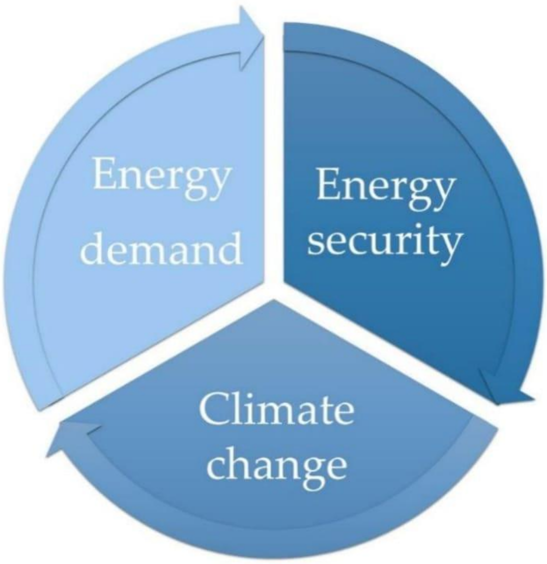

Figure:2

The production process of bioethanol involves several stages, including hydrolysis, fermentation, and product purification. Recent advancements in bioethanol production have focused on various technologies and processes to enhance its efficiency and yield.

Pretreatment of biomass: Pretreatment of biomass is an essential step in bioethanol production, aiming to separate its components and make it more accessible for subsequent conversion. Various pretreatment methods exist, which are used on the basis of which type of biomass is used for Bioethanol production. Effective pretreatment reduces biomass size, minimizes sugar loss, and maximizes lignin removal. The main goal is to disrupt the recalcitrant structures of the biomass to facilitate the release of sugars for bioethanol production. This step of Bioethanol production help in break down the lignin and disrupt the crystalline structure of cellulose, making the biomass more amenable to enzymatic and microbial reactions. The choice of pretreatment method is influenced by factors such as cost-effectiveness, environmental impact, and the properties of the specific biomass. Overall, the pretreatment of biomass is a critical step in the production of bioethanol from lignocellulosic materials, and the selection of the appropriate method depends on various factors including cost, environmental impact, and the specific characteristics of the biomass.

Detoxification: After the pretreatment step, bioethanol production from biomass requires a series of consecutive processes to obtain a final product, including detoxification, hydrolysis, fermentation, distillation, and dehydration. Detoxification aims to remove all the toxic compounds from pretreated biomass or hydrolysates, including fermentation inhibitors (such as furan aldehydes, aliphatic acids, and phenolic compounds) that could minimise the enzymes' efficiency and restrict microbial growth and activity during fermentation[95]. The most common methods to discard inhibitors from biomass and ensure higher bioethanol yield and productivity are, nowadays, various in situ strategies, including membrane extraction, solvent extraction, ion exchange, membrane bioreactors, adsorption, microbial adaptation, using microbial consortium or engineered microorganisms, and several other techniques that are tailored according to pretreatment, hydrolysis, and fermentation methods used in the ethanol production process. Detoxification may be performed separately or integrated into hydrolysis or fermentation.

Hydrolysis :

After the pretreatment and Detoxification stage is completed, raw material is subjected to enzymatic hydrolysis. This process is carried out to obtain fermentable sugars, pentoses, and hexoses from polysaccharides present in the pretreated lignocellulosic biomass. Mainly enzymes are employed to catalyse the hydrolysis of cellulose and hemicellulose (xylan), but also acids and alkalis can be used for this purpose .The enzymes capable of hydrolysing cellulose to glucose monomers are known as cellulases. They are multienzyme complexes consisting of mainly three various components, namely endo-1,4-β-D-glucanase (EC 3.2.1.4; breaks intermolecular bonds in cellulose randomly), exo-1,4-β-Dglucanase/exo-cellobiohydrolase (EC 3.2.1.91; removes monomers and dimers from the end of the glucose chain), and βglucosidase (EC 3.2.1.21; hydrolyses glucose dimers, cellobiose, and other short cellulose oligomers into glucose monomers). Complete hydrolysis of a native cellulose polymer into glucose monomers requires the synergistic action of all three components (Figure 3). [96].Cellulases are sourced from various bacteria and fungi. They are produced by aerobic, anaerobic, mesophilic, and thermophilic microorganisms. Cellulases producing microorganisms include bacterial genera of *Acetovibrio*, *Clostridium*, *Cellulomonas*, Cellvibrio, *Bacillus*, *Bacteroides*, *Erwinia*, *Ruminococcus*, *Streptomyces*, and *Actinomycetales genera* of Microbispora and *Thermomonospora*. Among fungal species, the most

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common source of cellulase is *Sclerotium rolfsii* and *Phanerochaete chrysosporium* species, as well as some species belonging to the genera of *Aspergillus, Caecomyces, Humicola, Neocallimastix, Oprinomyces, Penicillium, Schizophyllum*, and *Trichoderma* . Cellulose hydrolysis is difficult because the cellulose *microfibrils* are stabilised by internal and external hydrogen bonds and surrounded by hemicellulose polysaccharides (mannans and xylans) joined by covalent and hydrogen bonds; hence, the crucial role of the pretreatment stage emerges[97].

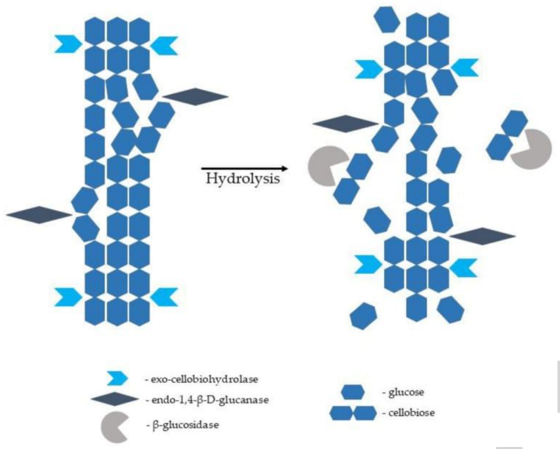

Figure 3 :Hydrolysis of biomass

Since hemicelluloses represent 10–30% of lignocellulosic biomass, their conversion to fermentable sugars is also vital for the high yield of bioethanol. Hemicellulose hydrolysis is easier than cellulose due to its more accessible amorphous structure. On the other hand, its more varied composition and structure, with multiple side chains containing various sugar types, requires a complex set of enzymes[98]. Two groups of enzymes are needed for effective hemicellulose hydrolysis: depolymerising core enzymes that can cleave the backbone and de-branching enzymes (so-called ancillary or auxiliary enzymes) that remove side chains posing steric hindrances to core enzymes, thus increasing the total yield of fermentable sugars obtained from lignocellulosic biomass. The core enzymes include β-1-4-mannosidases (EC 3.2.1.25), endo-1,4-β-mannanases (EC 3.2.1.78), endo-β-1,4-xylanases (EC 3.2.1.8), and xylan 1,4-β-xylosidases (EC 3.2.1.37), while de- branching enzymes are acetylxylan esterase (EC 3.1.1.72), α-Larabinofuranosidase (EC 3.2.1.55), β-glucuronidase (EC 3.2.1.139), ferulic acid esterase (EC 3.1.1.73), and p-coumaric acid esterase (EC 3.1.1-). Similar to cellulases, microorganisms are the source of enzymes for hemicellulose hydrolysis[99]. They include fungi, e.g., *Aspergillus niger, Aspergillus awamori, Trichoderma reesei, Penicillium wortmanii, Cochliobacillus carbonum, Agaricus bisporus*, and *other Aspergillus, Agaricus, Trichoderma*, and *Sclerotium genera*, and bacteria, e.g., *Thermotoga maritima, Clostridium thermocellum, C. cellulovorans, Thermobacillus xylanilyticus, Paenibacillus polymyxa cel44Cman26A, Cellvibrio japonicus, Caldibacillus cellulovorans, Caldicellulosiruptor Rt8b, Caldocellum saccharolyticum, Bacillus spp., and Streptomyces* spp.[100]. The synergistic action of various microbial enzymes ensures high sugar yield from lignocellulosic biomass, thus enhancing bioethanol production. The most critical parameters during biomass hydrolysis include solid loading, the concentration of sugars, enzyme loading, the shaking speed, hydrolysis time, the concentration of inhibitors, and the effect of various additives. **Solid loading**—High solid loading reduces hydrolysis installation costs and are necessary to obtain syrups with increased sugar concentrations (80–100 g/L), which determines economically viable distillation (i.e., the ethanol concentration in a fermented broth should be above 4% w/w). It was shown that sugar yield increases with increasing substrate load, but only to some point, after which it decreases. It is mainly because increased cellobiose and glucose concentrations inhibit enzyme activity. Additionally, high solid loading usually translates into a highviscosity broth, which causes several technical problems due to hampered mixing and impaired mass and heat transfer, affecting the efficiency of enzymes[101]. **Enzyme loading—**Increased doses of enzymes (or enzyme cocktails) enhance saccharification efficiency providing high glucose yield **. Shaking speed—** Optimising shaking/mixing speed is necessary to ensure optimal heat and mass transfer that translates into high glucose yield. Lower speed values result in poor mixing and decreased monosugar yields, while too high of a speed produces shearing forces that may destroy enzymes. **Hydrolysis time**—The long time required for complete hydrolysis limits the commercial production of ethanol from lignocellulosic biomass. Therefore, several approaches have attempted to shorten the process by enhancing hydrolysis efficiency, mainly using engineered enzymes/microorganisms or enzyme cocktails and optimising the parameters of the process. **Concentration of inhibitors**—Inhibitors produced during biomass pretreatment may slow down or even stop enzymatic hydrolysis. Therefore, the detoxification step (see Detoxification), performed before or during hydrolysis or selecting pretreatment methods producing only a limited amount of inhibitors, is crucial for the process. •Effect of various additives:Several different substances were successful as additives in the hydrolysis step to improve glucose yield, including polyethylene glycol (PEG)-based polymers (PEG 600, 4000, 6000), non-ionic surfactants (Tween 80 and Triton X100), non-catalytic protein (bovine serum albumin (BSA)) or novel chemical surfactants, such as Silwet L-77[102]. Their mode of action is based on blocking the interactions between lignin and enzymes, thus intensifying positive substrate-enzyme interactions and recovering cellulose hydrolysability. Enzymatic saccharification is the most challenging and relatively expensive stage in bioethanol manufacturing from

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lignocellulosic biomass, with costs estimated at 20–30% of the total production costs[103]. It has also been recognised as a techno-economical bottleneck in the whole process of biomass-to-ethanol bioconversion. Therefore, all crucial steps impacting the yield of fermentable sugars and total bioethanol require careful optimisation while maintaining minimum operational costs to make the production of lignocellulosic ethanol widespread and profitable.

Fermentation:

Ethanol Fermentation In the bioethanol production from lignocellulosic biomass, both hexoses (glucose, fructose, and sucrose) and pentoses are available for ethanol fermentation (xylose, mannose, galactose, and arabinose), resulting in the production of the respective number of ethanol and carbon dioxide molecules (Figure 4).

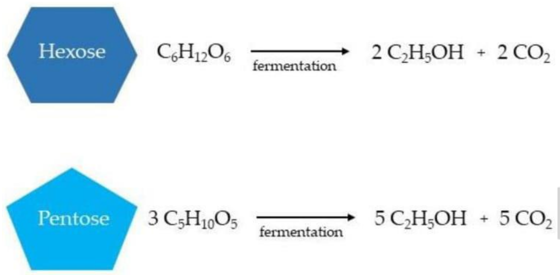

Figure 4: Fermentation of hexose and pentose

For glucose fermentation, industrial strains of Zymomonas mobilis and Saccharomyces cerevisiae are mainly used, owing to their high ethanol productivity and resistance to high ethanol concentration (up to 120 g/L). However, they are incapable of fermenting pentoses, which limits their use in ethanol production from lignocellulosic raw materials. Among microorganisms naturally fermenting pentoses are yeasts, such as Candida shehatae, Pachysolen tannophilus, and Pichia stipitis (recently reclassified as Scheffersomyces stipitis), and intestinal bacteria; however, the efficiency of the process is minor. Moreover, in the case of pentose-fermenting yeasts, large-scale utilisation is inhibited by their sensitivity to high ethanol concentration (over 40 g/L) and inability to ferment xylose at low Ph[104]. In addition, they require microaerophilic conditions and are easily inhibited in the presence of glucose (catabolite repression) and, in a mixed sugar broth, they usually utilise xylose only under glucose-limited conditions. Due to the lack of natural microorganisms for the efficient simultaneous fermentation of pentoses and hexoses, there is a growing interest in using engineering techniques for metabolic processes to construct organisms with the desired characteristics. Metabolic engineering aims to improve microbial activity due to changing enzymatic, transport, and regulatory functions using recombinant DNA technology. It includes analysing metabolic pathways, designing genetic changes, and creating recombinant cells with enhanced desired properties. The modification goal is to obtain a microorganism able to ferment all sugars in the biomass, tolerating stress conditions, showing high resistance to inhibitors, and producing a mixture of synergistic enzymes necessary for the complete hydrolysis of all lignocellulose carbohydrates. Among the most frequently modified microorganisms are *Saccharomyces cerevisiae, Zymomonas mobilis*, and Escherichia coli, but also other bacterial and fungal species were tested, including *Fusarium oxysporum, Thermoanaerobacter mathranii*, and *Corynebacterium glutamicum*[105]. Designing perfectly engineered microorganisms with the maximum conversion of monomeric sugars and enhanced tolerance to operational conditions will allow for economically feasible industrial production of bioethanol from lignocellulosic biomass. Another way to increase the fermentation efficiency is to use immobilised recombinant microbial cells. Immobilisation is placing intact cells on a suitable carrier using entrapment within a porous matrix, adsorption on the solid carrier surface, fixing to the carrier surface by covalent bonding or cross-linking, or encapsulation without altering their preferred catalytic activity. A carrier should be nontoxic, biodegradable, and cost-effective[106]. For yeasts cells, mainly Ca-alginate, carrageenan, cellulose, chitosan, silica-hydrogel, and pre-polymers are used as carriers. The sugar-to-ethanol conversion process can be conducted as a batch, fed-batch, or continuous fermentation, where the fed-batch mode in a stirred tank is the most frequently used in the industry since it provides the optimum conditions required for the microbial strain applied. Industrial biorefineries employ several fermentation technologies to increase ethanol yield and reduce production costs[107].

Separate hydrolysis and fermentation (SHF)—Hydrolysis and fermentation processes are conducted independently in different units. Carbohydrates from pretreated biomass are degraded to monosugars in a hydrolysis reactor and subsequently converted to ethanol in a fermentation unit. It is a time-consuming and costintensive process due to the long residence time needed for complete hydrolysis, high enzyme loading, and material costs required for two separate units, and its main drawback is endproduct inhibition .The fermentation strategies used to optimise the process[108]

Simultaneous saccharification and fermentation (SSF)—Hydrolysis and fermentation are carried out in the same unit, which improves hydrolysis rates, yields, and product concentrations compared to SHF due to the continuous removal of the sugars by the yeasts, which reduces the end-product inhibition of the enzyme complex. The main drawback is the difference in optimum temperature between saccharification and fermentation and enzyme inhibition by ethanol, microorganisms, and temperature in the reactor[109] .

Simultaneous saccharification and co-fermentation (SSCF)— Hydrolysis and fermentation are carried out in

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the same unit with concurrent co-fermentation of pentoses using pentosefermenting strains, which allows converting both hexoses and pentoses from lignocellulosic biomass, thus increasing ethanol yield. This process is suitable for xylose-rich biomass, such as hardwood and agricultural residues; however, the ethanol yield is lower compared to SSF[110].

Consolidated bioprocessing (CBP)—A single-step process where hydrolysis, fermentation, and enzyme production occur in the same unit. The method employs genetically modified microbes or microbial consortia (e.g., some yeast strains and Clostridium *thermocellum* have already been tested) capable of hydrolysing biomass with enzymes produced on its own and fermenting monosugars to ethanol. The strategy has the potential to revolutionise bioethanol production due to reduced costs for infrastructure and chemicals, making it economically beneficial and environmentally friendly. However, reaching an industrial scale is challenging because of low conversion efficacy, and it still requires further extensive research . Effective fermentation of monosugars obtained from lignocellulosic biomass is the next bottleneck in bioethanol production. Several factors might affect its efficiency, including temperature, time, pH, inoculum size, sugar concentration, solidto-liquid ratio, agitation rate, oxygen content, and rotation speed. Additionally, the operating conditions must be adjusted depending on whether the fermentation is conducted simultaneously or separately with saccharification, which is challenging and requires careful optimisation[111].

Distillation and Dehydration:

Distillation and dehydration are vital steps for obtaining fuelgrade ethanol from lignocellulosic biomass. Distillation allows for the effective separation of a component substance (such as ethanol) from a miscible liquid mixture (such as fermentation broth) through consecutive selective evaporation and condensation processes based on a difference in their volatilities. The water content in the post-fermentation mixture is very high, usually exceeding 80% of the dry weight. Therefore, concentrating ethanol up to 96% requires a huge amount of energy, which generates high costs. The first stage of the process is the so-called “drive away the alcohol”. The product (about 37% bioethanol) is then concentrated in a rectification column to a concentration of about 95% and finally dehydrated to a high-quality dry product which holds a minimum of 99.5% ethanol by volume[112] .Various methods for separating ethanol from a fermentation broth in bioethanol production have been developed, such as adsorption distillation, membrane processes, azeotropic distillation, diffusion distillation, extractive distillation, pervaporation, vacuum distillation, and chemical dehydration, differing in the technique employed, effectiveness and operational costs . Among them, membrane distillation and pervaporation are the most economically viable for bioethanol production. Membrane distillation is a method that allows for the reduction in the energy expenditure of the process of obtaining ethanol at the stage. During distillation, a membrane separates the fermenting solution from the distillate. Membranes that are used are flat or capillary, porous with gas-filled pores (porosity in the range of 70–85%), hydrophobic (not wetted by liquid), and with high thermal resistance. The process is feasible when there is a pressure difference between molecular components in the gas phase. Different types of membrane distillation have been developed, including contact, air-gap, vacuum, and sweeping gas membrane distillation. The main advantage of using a distillation membrane is the possibility of carrying out the process at a lower temperature. This eliminates the cost of heating the water to the boiling point of ethanol, thus reducing the total costs of bioethanol production. Other advantages of membrane distillation are the possibility of almost 100% retention of non-volatile compounds, lowering the process compared to conventional distillation, obtaining saturated solutions, and implementing durable artificial plastic installations (corrosion-free). Additionally, membrane distillation enables the continuous fermentation process with simultaneous ethanol stripping. Pervaporation is another type of membrane process that can be employed for obtaining anhydrous bioethanol on an industrial scale. This process uses the difference in ethanol concentrations on both sides of the asymmetric thick polymer membrane. The separation mechanism is based on the differences in the affinity of ethanol and water to the membrane (dissolving and diffusion capacity) and allows the final ethanol dehydration to be 99.8% [113].

XI. RENEWABLE RESOURCES USED FOR BIOETHANOL PRODUCTION

Renewable Resources used for Bioethanol production: •The New Webster's dictionary defines "renewable" as "replaceable or by human activity". Renewable resources are resources that are replenished by the environment over relatively short period of time. •Many renewable resources are used for producing of Bioethanol which are given below

Sugarcane: Sugarcane is one of the most widely used feedstocks for bioethanol production, particularly in countries like Brazil and India. It contains high levels of sucrose, which can be readily fermented into ethanol.

Corn (Maize): Corn is the primary feedstock for ethanol production in the United States. The starch present in corn kernels is converted into fermentable sugars through enzymatic hydrolysis, followed by fermentation.

Wheat: Wheat grains can also be used as a feedstock for ethanol production, although it is less common compared

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to corn and sugarcane. Similar to corn, the starch in wheat undergoes enzymatic hydrolysis to produce fermentable sugars. **Sorghum:** Sorghum is a drought-tolerant cereal crop that is used as a feedstock for bioethanol production in regions with arid or semi-arid climates. Its high sugar content makes it suitable for fermentation.

Barley: Barley grains can be utilized for ethanol production, primarily in regions where barley is a major crop. The starch in barley is converted into sugars for fermentation.

Cassava: Cassava, also known as tapioca or manioc, is a tropical root crop that is used for bioethanol production in countries like Thailand and Brazil. Its high starch content makes it a suitable feedstock.

Cellulosic Biomass: Cellulosic biomass includes various nonfood sources such as agricultural residues (corn stover, wheat straw), forestry residues, energy crops (switchgrass, miscanthus), and municipal solid waste. These feedstocks contain cellulose, hemicellulose, and lignin, which can be converted into sugars through pretreatment and enzymatic hydrolysis, followed by fermentation into ethanol.

Algae: Certain types of algae contain high levels of carbohydrates or lipids that can be converted into ethanol. Algae-based bioethanol production is still in the research and development phase but holds promise due to its high growth rates and potential for cultivation in non-arable land.

Other Biomass: Other biomass feedstocks such as woody biomass, agricultural residues (rice husks, sugarcane bagasse), and organic waste materials can also be used for ethanol production, particularly in the context of second-generation bioethanol production.

Molasses: Molasses, a by-product of sugar refining, is used as a feedstock for ethanol production in some regions. It contains fermentable sugars derived from sucrose.

XII. CHALLENGES AND FUTURE DIRECTION

There is also a lack of studies on a mix of waste raw materials for biofuel production, particularly in the second generation of bioethanol, which is involved in the use of food wastes. Additionally, the continuous bioethanol production systems require efficient air supply to improve yeast cell yield and concentration, which poses a challenge. The reduction of production costs of bioethanol is a significant ongoing challenge, and the development of the biorefinery concept is needed to make bioethanol more economically competitive than fossil fuels. Bioethanol production from lignocellulosic biomass faces several challenges, including the high cost of feedstock, low yield, and the need for efficient pretreatment methods. The use of food waste as a feedstock for bioethanol production is also being explored. Future directions in bioethanol production include the development of new and efficient pretreatment methods, the use of genetically modified microorganisms to increase yield, and the use of lignocellulosic biomass as a feedstock. The production of bioethanol from food crops such as corn and wheat is also being explored. The challenges and future direction for the bioethanol to jet fuel route are also being studied. Biorefineries will need to manage their own energy needs, produce low volume but highvalue products such as human food, chemical precursors, or medicinal compounds, and produce high volume low-value products such as fuels and animal feed to be highly profitable[114]. Technological improvement in pretreatment, enzymatic hydrolysis, fermentation, and distillation must be investigated further to increase the economic and environmental efficiency of lignocellulosic bioethanol production. The statical analysis demonstrated that raw material variability, chemical constituents are influencing factors for maximum glucose/ xylose recovery, which are directly proportional to ethanol yield (Andrade et al., 2017)[115]. Future direction of Bioethanol production: Nonrenewable fossil fuels such as coal and petroleum can't keep up with rising energy demands. Furthermore, global warming caused by CO2 emissions is now a serious environmental threat. This problem can be solved using bio-energy, as it is renewable, environmentally friendly, and can generate jobs. Bio-fuels such as ethanol, biodiesel, and biobutanol can be produced using different appropriate conversion methods (waste cooking oils, agricultural waste, algal biomass, municipal solid waste, and Lignocellulosic Biomass) which are present in limited amount[116]. The future direction of bioethanol Production is utilization of indigenous and native raw materials for bioethanol production. Use of continuous bioethanol production systems consisting of a cascade of continuous bioreactors. Continuous ethanol removal from broth to enhance bioprocess productivity. Increasing air supply to improve yeast cell yield and concentration in continuous systems of bioethanol production. •Biorefinery concept to produce low volume but high-value products such as human food, chemical precursors, or medicinal compounds, and high volume low-value products such as fuels and animal feed to be highly profitable[117]. The future direction of bioethanol production is likely to focus on several key areas:

Advanced Feedstocks: Increasing the use of non-food feedstocks such as lignocellulosic biomass, algae, and waste materials to improve sustainability and reduce competition with food production.

Technological Innovation: Continuously improving fermentation processes, enzyme efficiency, and biomass pretreatment techniques to enhance bioethanol yields and reduce production costs.

Biorefinery Integration: Integrating bioethanol production with other biorefinery processes to maximize

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resource utilization and produce value-added co-products such as biochemicals, bioplastics, and animal feed.

Sustainable Practices: Implementing sustainable practices to minimize environmental impacts, including reducing water usage, optimizing energy consumption, and mitigating greenhouse gas emissions throughout the production process.

Genetic Engineering: Advancing genetic engineering techniques to optimize feedstock characteristics, enhance microbial fermentation efficiency, and improve tolerance to environmental stresses**.**

Policy Support: Continued government incentives, subsidies, and regulations promoting the use of biofuels as a renewable energy source to drive investment and market demand.

Market Diversification: Expanding bioethanol applications beyond transportation fuels, such as in aviation, marine, and industrial sectors, to create new markets and revenue streams**.**

International Collaboration: Collaborating on research, development, and technology transfer initiatives globally to accelerate innovation, address regional challenges, and facilitate the adoption of bioethanol production technologies.

Circular Economy Initiatives: Embracing circular economy principles to minimize waste generation, promote resource efficiency, and maximize the reuse and recycling of by-products and residues from bioethanol production processes.

Public Engagement and Education: Engaging with stakeholders, raising awareness, and promoting the benefits of bioethanol production in reducing greenhouse gas emissions, enhancing energy security, and fostering rural development to gain public acceptance and support.

XIII. ADVANCEMENT IN BIOETHANOL PRODUCTION

Engineered microorganisms to increase bioethanol yield and productivity, as well as the development of novel biocatalysts and fermentation strategies. Additionally, there have been improvements in separation and purification methods for bioethanol extraction, aiming to make the production process more cost-effective. Furthermore, the biorefinery concept is being utilized to comprehensively utilize renewable feedstocks and produce biobased materials, which could help reduce the production cost of bioethanol and increase its economic competitiveness compared to fossil fuels and biofuels[118]. Advancements in bioethanol production have also been driven by developments in synthetic biology and genetic engineering, which have the potential to revolutionize the bioethanol industry. These advancements not only focus on enhancing bioethanol production but also on the possibility of producing other valuable chemicals, such as bioplastics and biofuels, in a single fermentation process, thereby increasing the economic viability of bioethanol production[119]. In summary, the production of bioethanol has seen significant advancements in process technologies, biochemical engineering, and genetic engineering, aiming to improve its efficiency, yield, and economic competitiveness compared to fossil fuels. There are many techniques used to produce maximum Bioethanol yield and utilizes minimum substrate as a nutrient source.

Genetic engineering: •This method is used to modify the biomaterial structure or the microorganism involved by inserting the gene of interest to enhance the process output. •This technique can provide multiple benefits like removing the pretreatment step, increasing sugar content, reducing the cost of cellulase enzyme, and providing co-utilization of sugars through engineered microorganisms. Lignin hinders the accessibility of cellulose and hemicellulose for bioconversion. Modification in the structure of lignin to reduce lignin content through downregulation of the enzyme required in the lignin biosynthesis route can eliminate the pretreatment step. Likewise, sugar content increase can be achieved by opting for the following approaches: diverting carbon from lignin production, modifying plant growth regulators, and delaying flowering. It has been assumed that suppressing flowering genes will increase biomass production. The price of the cellulase enzyme reduces up to 5 folds if plants are modified to express the enzyme. Also, expressing cellulase genes in yeast helps carry out simultaneous saccharification and fermentation (SSF). Many fermenting microbes (*S. cerevisiae* and *Z. mobilis*) do not utilize pentose as carbon, affecting overall ethanol yield. This limitation can be overcome by modifying the fermenting microbes. E. coli is an efficient *ethanologenic* microorganism, however, provides low yield due to generation of other organic compounds from sugars instead of ethanol. On modification, E. coli overexpressed the enzymes (alcohol dehydrogenase and pyruvate decarboxylate) that resulted in enhanced ethanol production. Chou et al. co-expressed the *ictB* and ***ecaA*** genes in *Synechococcus elongatus PCC7942* and obtained 202.7 mg/L ethanol production. *Zingaro et al*. expressed the *GroESL* gene in E. coli and increased its ethanol tolerance up to 6%.[120].

Adaptive evolution

This approach improves the microorganism's ability to survive under stressed conditions. In this method, the microorganism is cultured under specific conditions for a long time, i.e. from weeks to years, improving the phenotype of microbial species. High ethanol concentration is toxic to yeast and affects the cellular protein and plasma membrane fluidity, and impairs the transport system. The ethanol production rate can be

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enhanced by increasing the tolerance of the yeast Zhang et al. conducted adaptive evolution to enhance ethanol production in which *S. cerevisiae* was exposed to multiple stresses, i.e. freeze-thaw treatment, ethanol, and osmotic stress resulting in a robust strain that had high tolerance toward ethanol and osmotic pressure than the wild strain. Novelli Poisson et al. used an adaptive evolution methodology to improve the activity of *Scheffersomyces stipitis* for the production of ethanol. The strain was exposed to osmotic and ethanol stresses[120]. The results showed that the evolutionary S. stipitis strain could be used for ethanol production from non- detoxified hydrolyzate due to increased ethanol and osmotic tolerance. Yan et al. evolved *Z. mobilis* strain with improved inhibitor (phenolic aldehydes) tolerance by 6.3 fold and enhanced ethanol fermentation by 21.6% laboratory adaptive evolution for 198 days.[121].

Fermentation Optimization :

Adjust pH: Optimize pH levels to the range where the specific microbial strains used for fermentation perform best, typically between 4.0 to 6.0. Temperature Control: Maintain an optimal temperature range for microbial activity, usually between 25 to 35 degrees Celsius, depending on the strain. Nutrient Management: Ensure proper nutrient concentrations, including nitrogen, phosphorus, and trace minerals, to support microbial growth and ethanol production[122].

Oxygen Levels: Control oxygen exposure during fermentation to prevent oxidative stress, which can inhibit ethanol production.

Fermentation Time: Determine the optimal duration for fermentation to maximize ethanol yield while minimizing the formation of by-products[124]**.**

Mixed Culture Fermentation:

Utilize diverse microbial populations: Combine different strains of yeast, bacteria, or fungi with complementary metabolic capabilities to enhance ethanol production.

Synergistic Effects: Take advantage of synergistic interactions between microorganisms, such as cross-feeding or metabolic cooperation, to improve overall fermentation efficiency.

Species Selection: Choose microbial species that naturally produce high levels of ethanol or have the potential for metabolic engineering to enhance ethanol yield**.**

Metabolic Engineering Techniques:

Introduction of Non-GMO Pathways: Incorporate non-genetically modified metabolic pathways from other microorganisms or organisms to enhance ethanol production.

Endogenous Pathway Optimization: Modify native metabolic pathways within microbial strains to increase the flux towards ethanol production while minimizing the formation of byproducts.

Directed Evolution: Apply selective pressures, such as substrate limitations or environmental stresses, to encourage the evolution of microbial strains with improved ethanol-producing phenotypes without directly altering their genetic makeup.[122].

Process Control and Monitoring:

Real-time Monitoring: Implement sensors and monitoring systems to track key parameters during fermentation, such as glucose concentration, ethanol production rate, and biomass growth.

Feedback Control: Use gathered data to adjust fermentation conditions in real-time, optimizing process parameters for maximum ethanol yield.

Process Integration: Integrate fermentation with downstream processes, such as distillation or purification, to improve overall ethanol recovery and process efficiency. By using these strategies, it's possible to enhance bioethanol yield using microbial strains without maximum utilisation of biomass and not require specific conditions for producing Bioethanol.

XIV. ENVIRONMENT AND SUSTAINABILITY CONSIDERATION

The production of bioethanol can have both positive and negative environmental impacts. On the positive side, bioethanol is derived from renewable sources such as crops and waste materials, which can provide a more sustainable and secure energy supply. It has the potential to reduce our dependence on non-renewable energy sources, increase energy security, and decrease the impact of oil price fluctuations on the economy. Additionally, bioethanol can contribute to a more sustainable and diversified economy while promoting positive environmental outcomes. However, there are also potential environmental consequences, including the use of land and water resources, as well as emissions during production[123]. The use of food wastes in the second generation of bioethanol production is an area that requires more study, as it can have implications for the environmental impact of bioethanol production. The environmental impact of bioethanol production depends on various factors, including the feedstock used and the production methods employed. Because of its environmental benefits and renewable resources, Bioethanol, a renewable fuel, has grown in popularity[124]. Bioethanol production involves various environmental and sustainability considerations. Some key aspects include:

Carbon footprint: Bioethanol can offer a lower carbon footprint compared to fossil fuels if produced sustainably. However, the carbon footprint varies depending on factors

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such as feedstock type, cultivation practices, and production methods.

Feedstock selection: The choice of feedstock greatly influences the environmental impact of bioethanol production. For example, using food crops like corn or sugarcane can raise concerns about land use change and competition with food production. In contrast, using non-food feedstocks like agricultural residues, algae, or dedicated energy crops grown on marginal lands can mitigate these issues.

Land use: Sustainable bioethanol production aims to minimize land use change and avoid deforestation. This involves selecting feedstocks that do not compete with food crops or encroach on valuable ecosystems such as forests or grasslands.

Water usage: Bioethanol production requires significant water inputs, particularly in feedstock cultivation and processing. Sustainable practices aim to minimize water usage, optimize irrigation techniques, and implement water recycling and treatment systems.

Energy efficiency: Ethanol production processes, such as fermentation and distillation, consume energy. Sustainable production methods focus on improving energy efficiency through process optimization, use of renewable energy sources, and waste heat recovery.

Waste management: Bioethanol production generates various by-products and waste streams, including vinasse, stillage, and lignin. Sustainable practices involve managing these wastes effectively through processes like anaerobic digestion for biogas production, composting, or conversion to value-added products.

Biodiversity and ecosystem impacts: Sustainable bioethanol production considers the potential impacts on biodiversity and ecosystems, such as habitat destruction, soil erosion, and pollution. Practices like agroforestry, crop rotation, and integrated pest management can help minimize these impacts.

Social aspects: Sustainable bioethanol production also addresses social aspects such as land tenure, labor conditions, and community engagement. It aims to ensure equitable distribution of benefits, respect for local communities' rights, and adherence to labor standards.

XV. TECHNO - ECONOMIC ANALYSIS

A techno-economic analysis was conducted for a simplified lignocellulosic ethanol production process developed and proven by the University of Florida at laboratory, pilot, and demonstration scales. Data obtained from all three scales of development were used with Aspen Plus to create models for an experimentallyproven base-case and 5 hypothetical scenarios. The model input parameters that differed among the hypothetical scenarios were fermentation time, enzyme loading, enzymatic conversion, solids loading, and overall process yield. The minimum ethanol selling price (MESP) varied between 50.38 and 62.72 US cents/L. The feedstock and the capital cost were the main contributors to the production cost, comprising between 23–28% and 40–49% of the MESP, respectively. A sensitivity analysis showed that overall ethanol yield had the greatest effect on the MESP. These findings suggest that future efforts to increase the economic feasibility of a cellulosic ethanol process should focus on optimization for highest ethanol yield[125]. Due to the volatile nature of oil prices and environmental concerns, a great deal of attention has been placed on renewable biomass based fuels and chemicals to replace current petroleumbased products. Initial economic analyses performed on cellulosic fuel ethanol production cited conversion economics as the main issue to be addressed (Lynd et al., 1991), while other techno-economic models focused on optimization of operational costs (Nguyen and Saddler, 1991; von Sivers and Zacchi, 1995; Wyman, 1994). The National Renewable Energy Laboratory (NREL) published in 1999 a detailed analysis for lignocellulosic ethanol production and reported an ethanol production cost of 0.38 US$/L (1.44 US$/gal) (Wooley et al., 1999). A second report by NREL in 2002 with revised figures for equipment and installation costs, projected the required advances needed in key research areas with the aim to reach a MESP of 0.28 US$/L (1.07 US$/gal) in 2010 (Aden et al., 2002)[126]. Subsequent techno-economic analyses have made use of some of the parameters from the NREL report on the operation of an nth plant. Nevertheless, the reported MESP values have varied considerably from one study to the next based on the assumptions and process configurations (Aden and Foust, 2009, Chovau et al., 2013, Eggeman and Elander, 2005, Foust et al., 2009, Galbe et al., 2007, Hamelinck et al., 2005, Han et al., 2015, Kumar and Murthy, 2011, Macrelli et al., 2012; Sassner et al.). These differences have made it difficult to compare these studies (Chovau et al., 2013, Galbe et al., 2007, Sassner et al., 2008). The NREL report (Wright et al., 2010) was further revised with more representative values in 2011 and resulted in a MESP of 0.57 US$/L (2.15 US$/gal) (Humbird et al., 2011). Some of the significant contributors to the MESP of lignocellulosic ethanol include the cost of the feedstock, the ethanol yield, and the cost of cellulase enzymes (Chovau et al., 2013). However, the main contributor to the MESP in almost all cases seems to be the capital cost (Galbe et al., 2007). From various studies, it is clear that one way to lower the MESP is to simplify the process in order to reduce the capital cost of a lignocellulose-to-ethanol facility[127]. With this in mind, five research advances were identified that are required for process simplification: I).development of biocatalysts with improved resistance to hemicellulose toxins (eliminates the need for separate detoxification steps); II).replacement of sulfuric acid with the less aggressive phosphoric acid

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(eliminates the need for expensive metals or alloys); III).solving the mixing and pumping issues related to high fiber solids loading (simplifies material handling, reduces opportunities for contamination, and improves product yields); IV).limiting the use of chemicals to those that are nutrients for the biocatalyst and for ultimate use as a high nitrogen fertilizer (partial recovery of chemical cost through multiple usage); V).co-fermentation of hexose and pentose sugars in the same vessel (eliminates early liquid solid separation, fiber washing and detoxification of hemicellulose hydrolysate). Data from the biorefinery pilot plant, and laboratories were used to develop a techno-economic model for the construction of a 83 million liters per year (22 million gallons of ethanol per year) commercial plant in order to determine the economic feasibility of the process and to identify areas for further improvement. An experimentally proven case and 5 hypothetical scenarios were evaluated in which enzyme loading, enzymatic glucan hydrolysis, overall biomass-to-ethanol conversion, solids loading, and incubation time are varied. Scenarios were also compared in terms of heat demand, electricity, fertilizer production, and cost of ethanol production. The techno-economic analysis (TEA) of bioethanol production currently reveals a complex landscape. Feedstock costs vary widely depending on the region and type of biomass used, with corn averaging around $60-$80 per metric ton and sugarcane approximately $30-$50 per metric ton. Conversion technologies show promising advancements, with average ethanol yields ranging from 2.5 to 3.0 gallons per bushel of corn or 600-800 liters per metric ton of sugarcane. However, capital investment remains significant, with initial costs for establishing a bioethanol facility ranging from $1 to $3 per gallon of annual production capacity. Operating expenses, including labor and maintenance, typically amount to 30-40% of total production costs. Market dynamics play a crucial role, with ethanol prices fluctuating between $1.50 to $2.50 per gallon depending on factors such as crude oil prices and government policies. Overall, while bioethanol production holds promise as a renewable fuel source, optimizing technology and reducing production costs remain essential for long-term economic viability[128].

XVI. CONCLUSION

In conclusion, this review provides a comprehensive analysis of bioethanol, highlighting its pivotal role as a sustainable alternative energy source in the context of global energy transition. Through a meticulous examination of various aspects including production processes, utilization, advancements, and environmental implications, we underscored the significance of bioethanol in reducing reliance on finite fossil fuels and mitigating environmental impacts. The exploration of feedstock selection, pretreatment techniques, fermentation processes, and microbial strain development revealed the continuous advancements driving increased efficiency and cost effectiveness in bioethanol production. Moreover, the integration of renewable resources and the consideration of environmental sustainability further emphasize the positive attributes of bioethanol as a renewable energy solution. Despite the progress made, challenges such as feedstock availability, technological optimization, and environmental concerns remain, necessitating ongoing research and innovation. Moving forward, concerted efforts towards addressing these challenges, coupled with supportive policies and collaborative initiatives, are essential to harnessing the full potential of bioethanol and accelerating the transition towards a more sustainable energy future[129]. Because of its environmental benefits and renewable resources, Bioethanol, a renewable fuel, has grown in popularity. The production of bioethanol has several benefits in offsetting the general use of fossil fuels by increasing global supplies of liquid transport fuels in response to growing energy demand and improving energy security in regions devoid of fossil resource deposits. Thereby, bioethanol contributes to restricting worldwide dependence on fossil supplies and the petroleum industry, thus helping alleviate the energy crisis. Moreover, the transition from petroleum- to biomass-derived fuels reduces net carbon dioxide emissions per unit of energy produced and used, helping tackle anthropogenic climate change and its consequences for people and the environment. Therefore, intensive research has been conducted to develop new technologies that are efficient, economically viable, and universal for various biomass types, while being environmentally friendly. Although significant progress has been made in this field in the past decade, including the development of advanced engineered microorganisms or attempts to combine pretreatment, hydrolysis, and fermentation, or part of them into a single, more efficient step, there are still several gaps between novel findings and practical applications. Some of the most crucial challenges include the following: the selection of a suitable pretreatment strategy that is cost effective and does not impede the overall efficiency of enzymatic saccharification, the improvement of the anaerobic digestibility of biomass, limiting carbohydrate degradation and the generation of inhibitors during pretreatment to prevent conversion yield loss, downsizing the consumption of toxic chemicals, as well as energy

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and water, the improvement and application of novel biocatalysts that can enhance the efficiency of the saccharification process, increasing the efficiency of individual enzymes by designing enzymes with enhanced specific activity, thermal stability, and reduced end-product inhibition, and reducing the overall footprint of the process[130]. Detailed knowledge about the structure and composition of different biomass types is required, as well as the effects of individual pretreatment techniques on various biomass materials at the macro and molecular scales. Additionally, a thorough study of the interactions between biomass, microorganisms, products, and by-products generated during hydrolysis and fermentation at the molecular scale is necessary to establish optimal conditions for those processes. The existing knowledge is broad, but even more comprehensive interdisciplinary research is still needed to bring bioethanol production into a profitable and pervasive light for commercial use. However, it should also be remembered that transitioning from a laboratory to a commercial scale is extremely difficult and requires additional pilot-scale studies with optimisation and high financial expenditure. As for now, it seems that just using lignocellulosic biomass as a sustainable feedstock for bioethanol production does not guarantee a successful transition from petroleum-based to renewable biomass-derived energy. It seems that the strategy to utilise all components of the lignocellulosic complex by employing cost-competitive manufacturing processes designed with green chemistry is more likely to succeed. The future of this energy sector will be integrated biorefineries that produce both energy and value-added components for the chemical industry based on green chemistry principles with respect to the environment. This is achievable through enhancing the efficiency of all used materials and energy, reducing waste production and toxicity, and reusing resources and by-products[131]. Integrated biorefineries are gaining interest worldwide as they support the circular bioeconomy concept. However, greener processes and technologies are required, such as employing water-based reactions and environmentally friendly oxidants instead of materials and chemicals with high environmental burdens, or those using alternative energy-saving pretreatment methods, such as ultrasound or microwaves, which require time, effort, and f inancial investment. Since the 1970s, tremendous progress has been made to alleviate the use of fossil fuels. With the persistent passion of researchers worldwide, there is great optimism for the future of bioethanol from lignocellulosics. This review is meant to not only educate on bioethanol processes and their challenges, but also to illuminate novel and debatable research ideas. The Bioethanol is alternative energy source and have positive and negative impact on environment[132].

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