Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

ADVANCEMENTS IN LIQUID BIOFUELS (BIODIESEL, BIOETHANOL, BIOBUTANOL) FROM BIOMASS BIOREFINING: A REVIEW

Ifeanyichukwu Edeh and Ifeke Akpan
Department of Chemical Engineering, University of Port Harcourt, Nigeria

Abstact: Biodiesel, bioethanol, and biobutanol are considered alternative sources of energy obtained from biomass to replace or complement the conventional source of energy (fossil fuel), which is depleting due to overdependency. Aside from the depletion, it also causes serious environmental havoc due to the emission of large amounts of carbon dioxide (s) to the atmosphere. Thus, this chapter focuses on the production of some common replacements of fossil fuel (biodiesel, bioethanol, and biobutanol), which are sustainable, environmentally friendly, and economically viable with high energy content comparable to conventional fuels. It takes into consideration materials for production, the method of production, economic viability, and life cycle analysis. Novel approaches adopted for yield optimization during production and the mitigation of some drawbacks encountered during the production process are also considered.

Keywords: bioethanol, biobutanol, biodiesel, lignocellulose, hydrolysis, pretreatment, fermentation, techno-economic, life cycle assessment.

1. Introduction

The continuous quest for alternative and sustainable sources of energy keeps on pushing research boundaries for new energy sources that would not only replace conventional energy sources (fossil fuels) but also mitigate carbon footprint. Over the years, different biomass such as algae, cereals, oil plants, etc. have been tested to check their potential in generating energy (biofuel) suitable to be used as an alternative to fossil fuel. The use of this biomass for energy production is accompanied by enormous challenges, especially in terms of optimal production technique(s), which limit its ability to compete with conventional fuel. Nevertheless, researchers are developing novel strategies to enhance production, reduce production costs, and meet regulatory specifications.

Some of the areas considered for biofuel production advancement include feedstock, pretreatment method, fermentation techniques, production techniques, recovery method, and the catalyst used to aid in the production. Several methods have been developed over the years to maximize biofuel production; thus, this chapter focuses on the advancement in biofuel production with a major concern on biodiesel, biobutanol, and bioethanol.

2 Biodiesel

Biodiesel is a liquid fuel synthesized from both plant and animal lipids. In addition to being a sustainable energy source, it has the capability of replacing conventional fuel. Biodiesel is a renewable fuel that is also ecologically benign, efficient, and a replacement fuel. Thus, biodiesel is a viable alternative to conventional diesel and has the potential to lower the levels of environmental pollution caused by the use of conventional diesel.

The main raw material for biodiesel production is triglycerides (lipid) derived from various sources of biomass such as palm oil, castor oil, olive oil, and animal fat. Biodiesel has several desirable properties, which include non-toxicity, non-corrosive, and environmental friendliness. It has a high flash point, efficient combustion, low sulphur content, high cetane number, etc. Biodiesel is non-explosive and non-flammable, making it safer to store, handle, and transport than fossil diesel. For example, it has a flash point of 423 ºC, which is higher than that of the conventional diesel 337 ºC (Ling et al, 2014). Table 1 presents the standard specifications of biodiesel as stipulated by ASTM D6751 and EN14214 for B100.

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

Table 1. Specification for biodiesel based on ASTM D6751 and EN14214 (B100) Standards (Ramos et al, 2019)

Property	ASTM D6751	EN 14214
Density at 15 °C (kg/m3)	880	860 - 900
Viscosity at 40 °C (mm2/s)	1.9–6.0	3.5 - 5
Cetane number	Min. 47	Min 51.0
Acid value (mg KOH/g)	≤ 0.50	≤ 0.5
Pour point (°C)	-15 to -16	-
Flash point (°C)	≥ 130	≥ 101
Cloud point (°C)	-3 to -12	-
Water content (mg/kg)	≤ 500	≤ 500
Iodine number (g I2/100 g)	-	≤ 120

2.1 Raw Materials for Biodiesel Production

Because the cost of the raw materials accounts for up to 80 percent of the overall cost of producing biodiesel, these materials are essential. The ease of getting a high-quality feedstock in large quantities and the comparative manufacturing costs are mostly considered in selecting a feedstock to use. Previously, edible oils accounted for more than 95 % of the feedstock for the production of biodiesel. This has caused food crises in the world. The current trend in raw materials is geared toward the use of non-edible oil as a feedstock for the production of biodiesel. This helps to reduce the effect of food crises caused by the use of edible oils.

There are basically two primary categories of feedstocks for biodiesel production: edible oil and non-edible oil. Edible oils include, but are not limited to, soybean oil, peanut oil, sunflower oil, palm oil, coconut oil, and non-edible oils include rapeseed oil, Jatropha oil, Jojoba oil, and waste-cooking oil.

i) Algae Oil

Oil from algae is another promising source of oil for biodiesel production. Algae have about 20 % to 80 % oil content depending on the species. This oil is essential for the production of kerosene or biodiesel, depending on the procedure used for the production. Some identified species that are rich in oils are Euglena, Tribonema, etc. The oil composition differs from one species to another; some are very rich in saturated fatty acids, while some have low fatty acid content (Akubude et al, 2016).

ii) Municipal Sewage Sludge (MSS)

This is another promising feedstock for biodiesel production obtained from a wastewater treatment plant, which contains several amounts of organic and inorganic substances, including lipids, proteins, lignin, synthetic detergents, soap, carbohydrates, as well as various synthetic chemicals from industries. The disposal of this sludge to the environment causes pollution. Different strategies have been adopted for sewage sludge management, and one of which is '**reuse**'. The major challenge of using this as a feedstock is in lipid extraction due to the large water content.

2.2 Pretreatment methods for oils used in biodiesel production:

Oils used for the production of biodiesel come with some amount of contaminants, and as such, the percentage yield of the biodiesel is reduced during synthesis. Therefore, before employing it in biodiesel synthesis, pretreatment is necessary to reduce the level of contaminants. Below are some pretreatment steps for some of the oils used as biodiesel feedstock.

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

i) Free Fatty Acid Reduction using Acid

It is imperative to reduce the free fatty acids of the oil whenever the percentage is high to prevent side reactions like the saponification reaction. Commonly used acids for this include H_2SO_4, H_2PO_3, and HNO_3. The reaction is called esterification. The use of sulphuric acid (H_2SO_4) was studied by Hayyan et al. (2011) for reducing free fatty acid in palm oil sludge. The experiment used a temperature range of 40 °C to 80 °C and a methanol to oil ratio ranging from 6:1 to 14:1. Reaction times ranged from 33 – 120 min. The results demonstrated that under ideal circumstances of an 8:1 methanol to oil ratio, a temperature of 60 °C, and a reaction duration of 60 min, 0.75 weight percent H_2SO_4 was able to decrease the free fatty acid concentration from 24 to 2 weight percent.

ii) Free Fatty Acid reduction using Glycerolysis

Due to corrosion of equipment and excessive alcohol consumption caused by acid used to reduce free fatty acid, it is critical to develop alternative techniques of free fatty acid reduction. Glycerolysis is one of these ways. The process of glycerolysis converts free fatty acids to glycerides, which then react with alcohol to produce monoglycerides, diglycerides, and triglycerides.

2.3 Biodiesel Production Techniques

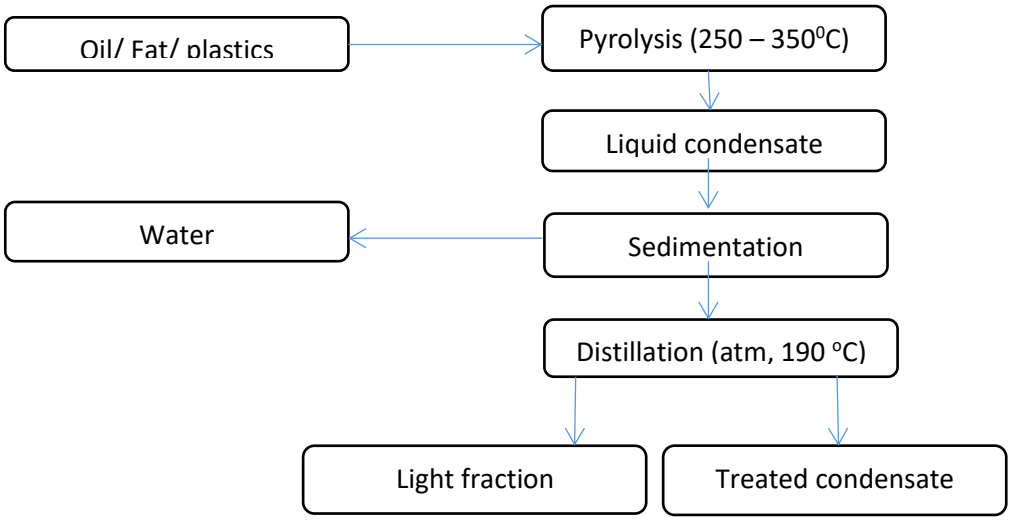
There are several methods used for the production of biodiesel, depending on the conditions of the raw materials, available resources, and technology. Most commonly used methods include microemulsion, transesterification, and pyrolysis.

a. Microemulsion

Microemulsion is an equilibrium state process and it involves the distribution of an optically isotropic microstructure with a dimension between 1 and 50 nm, which forms as a result of combining two immiscible liquids and one or more active substance(s) in a right proportion. Once the microdroplets are evenly distributed, the system becomes thermodynamically stable with low viscosity. In biodiesel production, the components include vegetable oil, alcohol, water, and a catalyst. The alcohol is mostly methanol or ethanol, acts as a surfactant/ emulsifier to allow miscibility of the other components. Biodiesel is produced by blending the different components in the right proportion. The major problem associated with biodiesel produced via this method includes incomplete combustion and accumulation of carbon in the engine, which may lead to nozzle failure.

b. Pyrolysis

In pyrolysis, a catalyst may be used; it involves heating the complex structural hydrocarbons in an oxygen-free environment to break the polymer and generate simpler molecules for biodiesel production. In the production, the feedstock (oil or fat) is first converted to bio-oil, and the bio-oil is then upgraded via hydrotreating or hydrocracking to obtain biodiesel. In hydrotreating, hydrogen reacts with oil to remove traces of sulfur and oxygen, while in hydrocracking, hydrogen is added to the bio-oil to create smaller chain hydrocarbons to meet biodiesel specifications. Figure 1 shows the thermal cracking route for biodiesel production.



Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

Figure 1. Schematic diagram for thermal Cracking route for biodiesel production

This method requires minimal application of technology for the production process. Catalysts like alumina or zeolite are used for this method. The pyrolysis process for biodiesel production is consistently carried out between 250 ºC and 350 ºC reaction temperature. Biodiesel is produced by heating oil in a reactor, which then condenses the resulting vapour. This method is useful for lowering the biodiesel's density and viscosity, two important factors that influence biodiesel in internal combustion in diesel engines.

c. Transesterification Reaction

Transesterification or alcoholysis, glycerol, and Fatty Acid Alkyl Ester (FAAE) are produced when triglycerides of fatty acids combine with alcohol in a nucleophilic way. It is a three-step reaction. First, the triglyceride is reduced to diglyceride; followed by the reduction of diglyceride to monoglyceride; lastly, the monoglyceride is converted to glycerol. Three molecules of ester are produced in the overall process since ester is created at each stage. A catalyst, which may be a base or an acid, an enzyme, a bifunctional catalyst, or any combination thereof, is used to speed up the rate of the reaction. The amount of free fatty acids in the oil determines which catalyst can be used. The production process involves the mixing of alcohol and catalyst, and is stirred while heating, thereafter poured into the oil in the reacting vessel, where the reaction occurs. The products obtained are subjected to separation for biodiesel recovery. Figure 2 shows a schematic diagram of different stages involved in the production of biodiesel via transesterification.

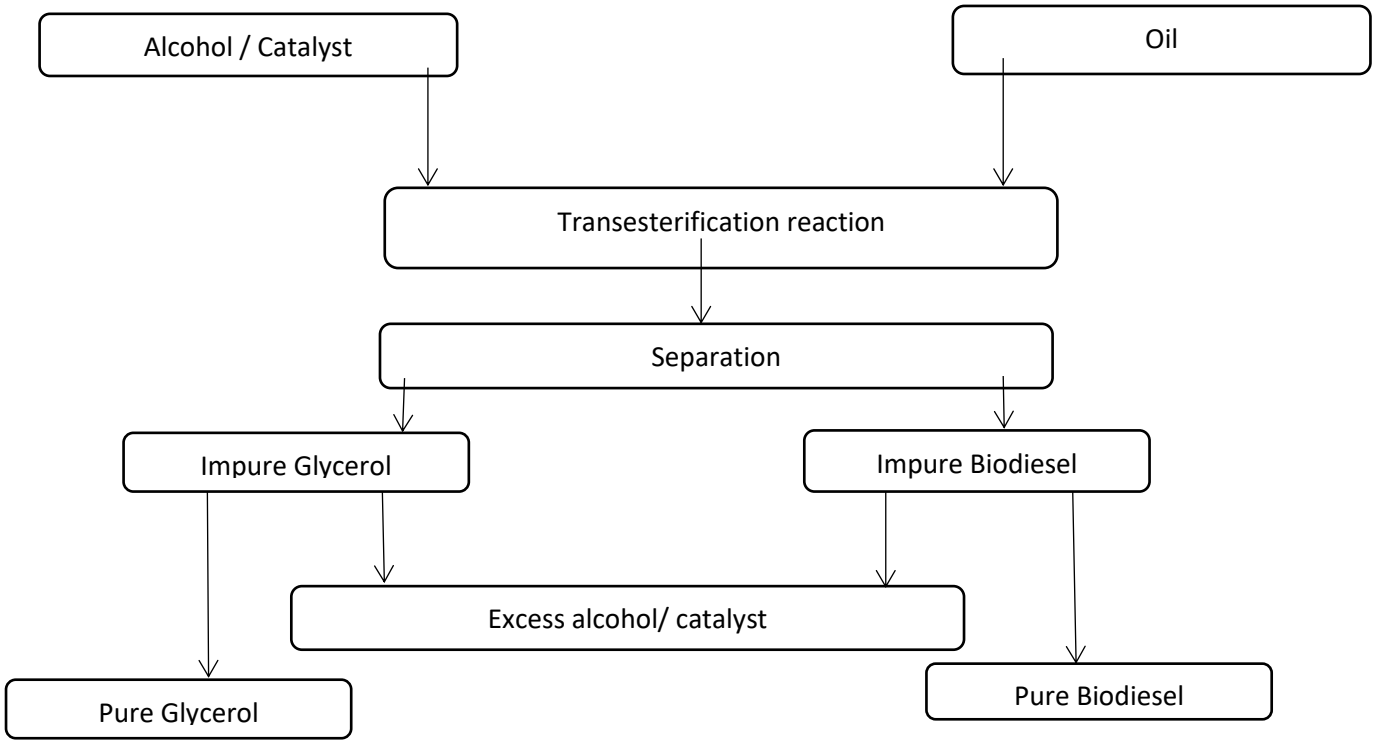


Figure 2. A schematic diagram of the transesterification reaction

During biodiesel production via transesterification, other side reactions also take place depending on the composition of the fatty acid or the type of catalyst used. If the triglyceride of high free fatty acid content is used with a base catalyst, the saponification reaction would

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

occur, or when an acid catalyst is used, the esterification reaction would take place before transesterification.

2.4 Technological Advancement in Biodiesel Production

The traditional transesterification reaction has some limitations, such as high cost of production, low efficiency, etc. To tackle these challenges, some advanced methods have been developed, including supercritical fluid transesterification, microwave-assisted transesterification, and ultrasonic-assisted transesterification.

i) Ultrasonic-assisted transesterification

In this method, ultrasonic waves are used to cause cavitation formation and the collapsing of the microbubbles within the reacting medium. This cavitation increases the rate of mixing of the reactants, causing mass transfer, which subsequently increases the rate of reaction. It is a very effective method when high-viscosity feedstock is used.

ii) Microwave-assisted transesterification

In this method, microwave radiation is used to maintain uniform and rapid heating of the reacting vessel. This reduces energy consumption compared to the traditional transesterification reaction. The overall yield of the reaction increases with less cost of production. It can easily be integrated into existing reactors with promising scale-up potential.

iii) Supercritical fluid transesterification

In supercritical fluid transesterification, alcohol is used at its supercritical state (temperatue between 200 ^{o}C and 400 ^{o}C, pressure of about 200 bar). At this state, it exhibits both liquid and gaseous behavior, making it to be hyperreactive. This method does not require the use of catalyst. The use of this method has several advantages which includes fast reaction time, no separation of catalyst, high yield and the capacity to produce biodiesel from feedstock with high free fatty acid. (Rathnam et al, 2020)

2.5 Reagents used for bioodiesel production

a. Alcohol

One of the key reactants in biodiesel production is alcohol. Based on the amount of functional groups connected to the main chain, alcohols are classified into three basic categories: primary, secondary, and tertiary. The primary alcohols like methanol and ethanol are the most commonly used in biodiesel production, while other alcohols have also been studied such as branch chain alcohols and other lower chain alcohols such as propanol, butanol, and isopropanol.

b. **Triglyceride**

Triglycerides used in the production of biodiesel are obtained from different sources such as (edible and non-edible feedstocks). Examples of primary sources include olive, palm fruit, babassu, linseed, lard, tallow from beef, grease, etc. These oils contain fatty acid either of same chain length or different chain length. Example of triglyceride include palmitic acid, oleic acid, linolenic acid, and stearic acid. Proper choice of the type of oil to be used for biodiesel production is very important as it influences the energy content of the biodiesel and the economic viability.

2.6 Factors affecting biodiesel production via transesterification

These include reaction temperature, reaction time, methanol to oil ratio, mixing rate, water impact, and free fatty acid content.

i.) Alcohol to Oil Ratio

In biodiesel production, alcohol to oil ratio is essential as it determines the yield. For example, the alcohol-to-oil ratio is set at 6:1 when using a base catalyst. Because transesterification is a reversible process, a significant quantity of methanol is required to maintain product-side balance. The equilibrium shift towards the product would be enhanced by increasing the methanol-to-oil ratio. Reaction rate and percentage yield are unaffected by increasing the ratio above a certain critical threshold (Yaqoob et al, 2020). When working with oil feedstock that has a high free fatty acid content, it is necessary to use more alcohol than catalyst when using an alkali catalyst for neutralisation. However, using too much alcohol can raise production costs due to purification and alcohol removal.

ii.) Temperature

Among other factors influencing biodiesel production via transesterification reaction is reaction temperature. Depending on the oil's properties and the catalyst's type, the transesterification reactioon may often occur at a

range of temperatures. It may happen at room temperature or close to the alcohol's boiling point, depending on the oil source.
As the temperature rises, the oil's viscosity decreases, which leads to increase in the reaction rate and decrease in reaction time. Therefore, a higher reaction temperature would facilitate oil-alcohol miscibility. However, the temperature must be carefully controlled so as not to go beyond the reaction's maximum value; otherwise, parallel reactions would occur.

iii) Reaction time
During the transesterification reaction, there is a varying trend in percentage yield as the reaction time increases. At the begining, the reaction rate is relatively slow because of less solvent dispersion and incomplete mixing of the reactants, during this stage the reaction occurres at the outer surface of the oil and triglyceride but with time agitation increases causing more reactants interaction which bring about the subsequent increase in yield. But beyond certain threshold time, reaction start to decrease and reverse reaction may occur.

iv) Amount of Catalyst
The catalyst used in the transesterification reaction has a significant impact on the biodiesel production. Using large quantity of catalyst increases the available active sites where reaction will occur, thus, the rate of reaction and yield are both increased. The produced biodiesel may have a high viscosity if large amount of catalyst isly used. Therefore, in order to avoid the high cost of purification, it is essential to maintain a controlled amount of catalyst. Generally, the biodiesel yield is increased when the catalytic loading is increased, up to a point where it begins to decrease.

v) Water and FFA Content
When choosing the reaction pathway to use during transesterification, the amount of water and free fatty acids in the reactants must be considered. In order for the reaction to take place, the feedstock must adhere to certain limits for both water and free fatty content. To prevent the saponification reaction, for instance, base-catalysts may only be employed in oils with a free fatty content of less than 1 %. Similarly, oils that contain a lot of water may result in ester production when an acid catalyst is used, but water or free fatty acid removal is not necessary for the transesterification reaction at supercritical conditions.

vi) Agitation Degree
Oil and alcohol do not completely mix with one another when they are combined; the reaction takes place at the boundary between the two liquids. First, transesterification reaction is therefore slow because of this. The transesterification process is accelerated by allowing the oil and solvent to contact, which is made possible through agitation. According to Tabatabaei et al. (2019), using a moderate agitation speed of 400 rpm yields the best result. It has been shown that reaction rates are slowed with slow agitation speeds, but soap formation takes place with high agitation speeds due to irreversibility.

2.7 Reactors used for Biodiesel Production
Reactors are equipment that provides enabling conditions for chemical reactions to take place. In a reactor, reactants are transformed into products after being subjected to the required conditions. When designing a reactor, many factors are considered. These include, but are not limited to, the nature of the reactant(s), method by which the reactant(s) are to be charged in, and the product(s) removed, materials of construction, type of stirring system, hydrodynamic, intake and discharge of heat, and size of the reactor. This is to ensure safety, productivity, and the economy of the process.
In biodiesel production, the reactor design (type) used is determined by whether the production process is considered to be batch or continuous. Some reactors used for biodiesel production include batch reactors, fixed bed reactors, continuous stirrer tank reactors, bubble column reactors, hybrid catalytic plasma reactors, membrane reactors, microchannel reactors, and reactive distillation.

2.8 Techno-economic analysis of biodiesel
Techno-economic analysis is an effective tool used in assessing the economic feasibility of different processes relating to biodiesel production. A comprehensive techno-economic analysis encompasses the cost of different feedstock, the intended production method to be adopted, and the scalability of such a process for industrial purposes. The technical aspect of the analysis involves the development of the process flow diagram, comprehensive material and energy balance calculations

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

using simulation software such as Aspen Plus, while the economic aspect development of the capital and project cost estimation, discounted cash flow, and determination of the minimum biodiesel selling price.

In biodiesel production, the major costs are those of feedstock and technology. The choice of feedstock and technology impact on the entire process cost. Using non-edible feedstock with a base-catalyst in the transesterification reaction is more effective in cost reduction. Advanced technologies like microwave, ultrasonic, and superfluid transesterification, though producing high yield, are capital-intensive (Akpan et al, 2023)

2.9 Life-cycle analysis of biodiesel

Life-cycle analysis is an important tool for assessing the environmental impact of products and processes involved in the production process throughout the entire life of the product. The LCA tool helps in the identification of potential impacts during a process design and for decision-making to improve the process before scaling up for commercialization. Biodiesel LCA involves the evaluation of material and energy input, output, and the subsequent environmental impact of cultivating the feedstock to the emission of gases in an internal combustion engine. Biodiesel LCA involves four stages: feedstock production, feedstock transportation and processing, production of biodiesel, and utilization of biodiesel in internal combustion engines. The analysis must also take into consideration input and output at each stage of the process.

Biodiesel LCA indicates that it has the capacity to reduce greenhouse gas emissions and global warming substantially, and hence help in the protection of the ozone layer from depletion due to low carbon footprint (Nogales-Delgado,2025).

3 Bioethanol

Bioethanol is a liquid biofuel obtained from biomass (plantain plant waste, sugar cane bagasse, forestry residues, paper and pulp waste, etc) through different processes, which include pretreatment, hydrolysis, fermentation, and purification. These feedstocks are classified under different generations, which include first generation (edible feedstock), second generation (lignocellulose), third generation (algae), and fourth generation (the use of modified microbes like E. coli). Many countries around the globe are currently producing and using bioethanol for different purposes, with the United States leading in 2021 with 1,435.8 petajoules of production (OECD, 2022). Other countries like Brazil, Canada, the European Union, and China are also major producers of bioethanol. In 2023, world production capacity stood at 106.76 billion liters, and it is estimated to reach 137.27 billion in 2028 (Global Bioethanol Market Report, 2023).

Bioethanol is a potential alternative to conventional gasoline and can be used either directly or blended with conventional gasoline, which helps in the reduction of greenhouse gas emissions and overdependence on conventional gasoline. The direct form is classified as (E100), while the blended forms are E85 and E10. If E100 is to be used in an existing internal combustion engine, the timing system has to be modified. However, the use of bioethanol (E100) is challenging, with difficulty in starting the engine at a low temperature (poor cold-starting property due to the high heat of vaporization of bioethanol). The blended forms (E85 and E10) bioethanol do not require modification of the engine; rather, they help in improving the ignition and engine performance. Advantages of bioethanol include a high-octane rating resulting in increased engine efficiency and performance, broad flammability, higher compression ratio, and comparable energy content (Carrillo-Nieves, 2019). The major disadvantage of bioethanol is the high cost of production, which results from the use of some feedstock, enzymes, detoxification, and ethanol recovery. Nevertheless, efforts have been made to produce bioethanol from low-cost feedstock (lignocellulose) through the use of modified processes.

3.1 Bioethanol production from lignocellulose

Lignocellulose biomass is a second-generation feedstock for bioethanol production. It is non-edible, not expensive, and is readily available around the globe. The lignocellulose energy consumption globally stands at about 40% and the annual output of about 10 -50 billion dry tons. Its common sources include barley straw, cotton stalk, banana and plantain plant waste, sugar cane bagasse, forestry residues, paper and pulp waste, etc. (Khaliq et al., 2020). Lignocellulose biomass comprises 25 to 30 % hemicellulose, 40 to 50% cellulose, 15 to 20% lignin, and some traces of pectin, nitrogenous compounds, and inorganic compounds (Mori et al, 2015). Cellulose is a linear syndiotactic (arrangement of side chain along the polymer backbone in an alternative

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

configuration) polymer made up of glucose linked together by β-1, 4-glycosidic bonds. Its peculiar polymer chain arrangement makes it a stable compound. Lignin is a 3-D heterogeneous polycrystalline mesh polymer that belongs to polyphenol compounds. It inhibits hydrolysis due to its complex hydrophobic polymer structure. Hemicellulose is a mixture that comprises polysaccharides with straight and branched chains. The polysaccharide in hemicellulose has a low degree of polymerization, which makes it easy to degrade to monosaccharides such as dextrose, fructose, xylose, mannose, galactose, etc. Figure 3 shows lignocellulose biomass and its components.

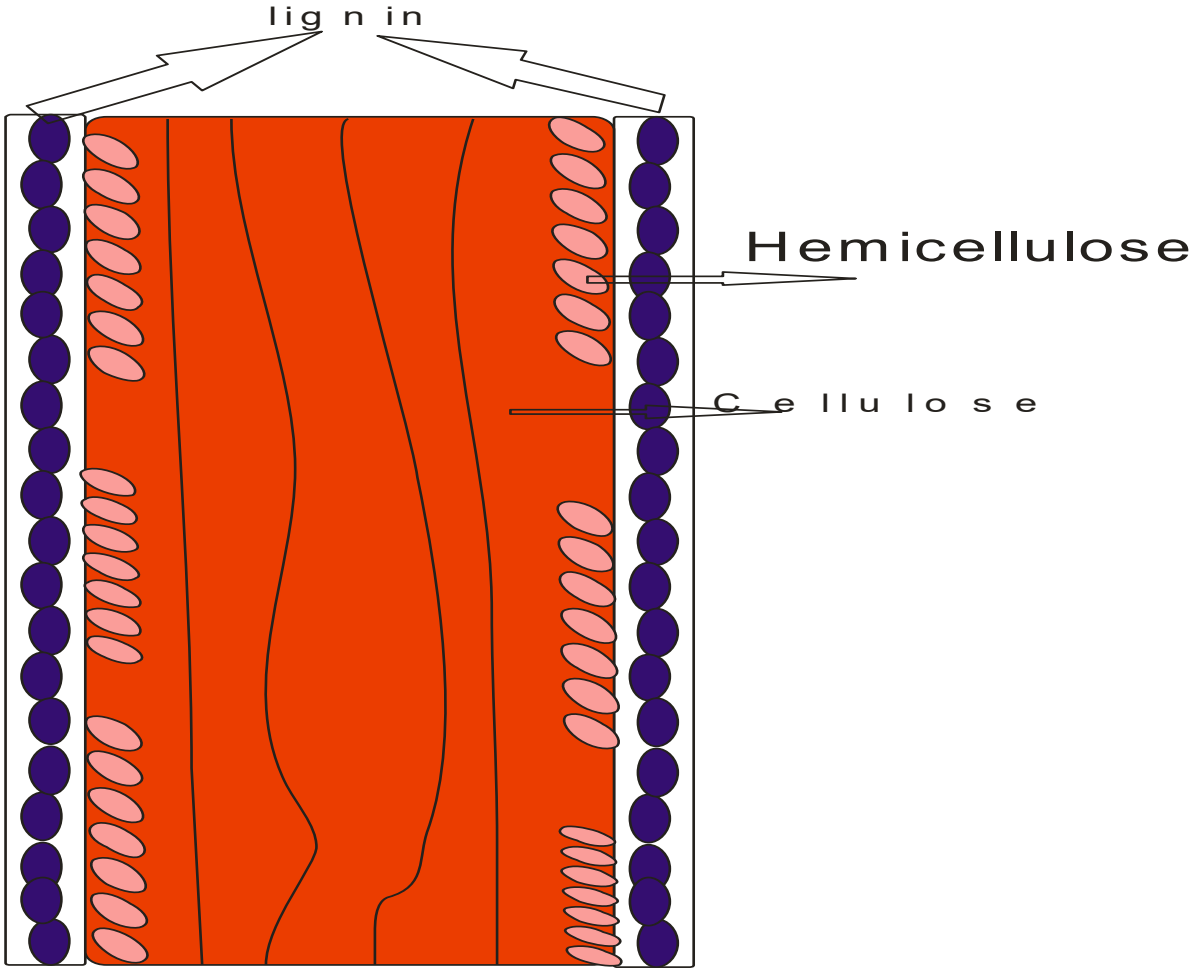


Figure 3. Lignocellulose biomass and its components

The lignocellulose structure poses challenges in its development for utilization, especially for bioethanol production due to the presence of lignin, which surrounds the entire structure of the cellulose and hemicellulose; thus, this necessitates proper pretreatment of the biomass to make it susceptible to microorganisms, enzymes, etc., to act on it. The following steps are involved in the conversion of the biomass to bioethanol: pretreatment, saccharization/hydrolysis, fermentation, and separation/purification.

3.2 Pretreatment

Pretreatment of lignocellulose is the most expensive stage in bioethanol production. It accounts for about $0.3/gallon of ethanol produced (Edeh, 2020**).** Various pretreatment methods have been developed in the last 80 years for efficient conversion of lignocellulose to bioethanol in view to minimize the cost and mitigating environmental impact. A good pretreatment method maximizes the efficiency of the hydrolysis process, which will in turn increase the yield of monomeric sugar, minimize carbohydrate degradation, and reduce inhibitor formation in the by-product (Procentese et al, 2017).

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

Some pretreatment methods include biological, chemical, physicochemical, physical, ionic liquid, and deep eutectic solvent pretreatments. Pretreatment of lignocellulose is essential because it helps to unwind the complex polymer matrix, which consists of lignin, hemicellulose, and cellulose. This exposes and increases the accessibility of enzymes to work on the cellulose during the hydrolysis stage.

i) Physical Pretreatment

Physical pretreatment involves the reduction of the size of the biomass after dehydration it including extrusion, irradiation via microwave, and size reduction.

a.) Size reduction

There are different methods of size reduction, and these include milling, grinding, coarse size reduction, chipping, and shredding are used to improve the digestibility of the lignocellulose during hydrolysis, which decreases the degree of polymerization, crystallization while increasing the surface area of the biomass for a faster rate of hydrolysis.

b) Extrusion

This method is designed to expose the biomass active sites for accessibility of the enzyme during hydrolysis. It involves using operating conditions like high shear, rapid mixing, less resident time, no furfural, no conditioning, and moderate barrel temperature. Extrusion pretreatment is very important because it can be applied on an industrial scale and it is environmentally friendly.

c) Microwave (irradiation)

This pretreatment makes use of an electromagnetic field to disrupt the lignocellulose polar bond by vibrating the structures until it is energized. As a result of the vibration, the complex polymer structures are broken down for easy attack by the enzyme. This pretreatment method is essential because of low energy demand, less inhibitor formation, ease of operation, high heating value, and degradation of the structural organization of cellulose fraction.

ii) Chemical Pretreatment

Chemical pretreatments are used to disrupt and remove lignin or hemicellulose from the polymer mixture. It includes the use of acid, alkali, organic acid, and oxidative delignification. The use of this method is highly selective depending on the type of biomass processing. It is very effective, though it involves harsh operating conditions which may affect the end-product, and it is not environmentally friendly.

a) Acid Pretreatment

There are two types of acid pretreatment: dilute acid and concentrated acid. The use of dilute acid leads to the formation of inhibiting by-products, though very useful in bioethanol production on an industrial scale. Depending on the end-product needed, two types of operating conditions are used for dilute acid pretreatment: temperature less than 120 ^{o}C for a long duration between 30 to 90 min, and high temperature more than 180 ^{o}C for a short duration between 1 to 5 min, respectively. Before further treatment of the biomass, the inhibitors produced as the cause of the acid treatment must be removed to make the process economically viable. Some of the acids that are used include phosphoric acid, hydrochloric acid, nitric acid, dilute Sulphuric acid, lactic acid, acetic acid, maleic acid, and peracetic acid. One major disadvantage of this method is corrosion of the reactor by acid (Lee and Jeffries, 2011).

b) Alkaline Prettreatment

Unlike acid pretreatment, alkali pretreatment operates at ambient temperature and pressure to disrupt the lignin complex structure into fractions (Refaat, 2018). Some alkali used for pretreatment includes hydroxyl derivatives of sodium, calcium, ammonium, and potassium salts. The solubility of hemicellulose and cellulose in alkali solution is less than that of acid solution. Comparatively, alkali pretreatment operates in less harsh conditions than acid, but when longer operating time is required, a higher temperature is recommended.

c) Organosolv Pretreatment

The use of organic solvents also proved to be effective in lignin deconstruction for enzyme accessibility during hydrolysis. Examples of organic solvents used are acetone, ethanol, methanol, glycol, ethylene, etc. The application of an organic solvent requires a catalyst for the process to be effective. Examples of such catalysts include lime, sulfuric acid, sodium hydroxide, ammonia, etc., aside from lignin, which is a valuable by-product during this process, syrup of hemicellulose C_5 and C_6, and cellulose fraction are also produced (Agbor et al,

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

2011). Due to the low boiling points of organic solvents, the process requires high pressure, which is a major drawback for the use of organic solvents; however, bioethanol produced from this process is of high quality.

iii) Biological Pretreatment

This is the application of a microorganism to degrade lignin in biomass. These microbes are capable of disrupting the cell wall of the biomass, and this promotes accessibility of the enzymes during the hydrolysis step. Some microbes used for this process include white rot, brown rot, soft rot fungi, and bacteria. Biological pretreatment does not produce unwanted products like chemical and physical pretreatment. It does not require the application of acid or base or harsh reaction conditions like high temperature or pressure; thus, it requires ambient conditions. Some lignin-degrading enzymes present in microbes include leccases, polyphenol oxidases, lignin peroxide, etc. The main disadvantage of this treatment method is a very low process rate, which result in a long residence time of reaction.

iv) Physicochemical method

This is the combination of both chemical and physical pretreatment methods to deconstruct the complex lignocellulose structure. It includes methods like steam explosion, CO_2 explosion, liquid hot water, ammonia fiber explosion, and wet oxidation.

a) Wet oxidation

Pretreatment using the wet oxidation method required subjecting the biomass to a temperature between 170 – 200 °C and high pressure between 5000 -200kpa for 10 – 15 min. This condition degrades the lignocellulose and produces fewer inhibitors, disrupts the lignin matrix, and eliminates lower-grade cellulose from the polymer matrix. This provides an enabling environment for the enzyme to work on the cellulose during the hydrolysis stage. Nevertheless, the common drawback of this method is the high energy requirement which increases the overall production cost. This method is very suitable for biomass with high lignin residuce. The overall efficiency of this method depends on using appropriate temperature, oxygen pressure and suitable resident time for the reaction. In this method, water under high temperature act as an acid to catalyze hydrolytic reaction. Hemicellulose are broken down to pentose monomer, lignin undergoes oxidation while cellulose remain neutral in this process.

In wet oxidation, chemical agents like sodium carbonate and alkaline peroxide help to promote the rate of reaction by decreasing reaction temperature and improve hemicellulose degradation.

b) **Carbon dioxide Explosion**

In carbon dioxide explosion method, CO_2 is subjected to supercritical conditions which make the gas to act as a solvent to disrupt the lignocellulose polymer matrix. The gas at supercritical conditions is made to pass through the vessel containing the biomass, the vessel is maintain at high temperature for several minute, at this high temperature, the gas enter the biomass to form carbonic acid which dissolved hemicellulose. The high pressure of the gas disrupt the biomass structure which promote enzyme accessibility (Hendrik eta al, 2009). The used of CO_2 explosion is depends on the type of biomass treat; it can only function well with biomass that has high moisture content. This method is very effective because it produces fewer inhibitors and eliminate lignin in non-harsh conditions (non-acidic and without corrosion of the vessel).

c) Ammonia Pretreatment

Ammonia pretreatment include Ammonia fibre explosion, soaking aqueous ammonia and ammonia recycled percolation. This method uses ammonia to pretreat lignocellulose.

In ammonia fibre explosion, lignocellulose is heated with liquid ammonia at a temperature between 60^0C °C - 100^0C and pressure between 250 – 300 Psi for a few minutes. The treatment causes dilation and phase change of the biomass cellulose crystalline, which promote reactivity of leftover carbohydrate after the pre-treatment (hydrolyses stage). Some operating parameters required for ammonia fiber explosion include appropriate ammonia loading, good water loading, and temperature. For the soaking aqueous ammonia method, the biomass is soaked in ammonia in a batch reactor at 30^0C – 60 °C for some minutes, depending of the nature of the biomass (Agbor et al., 2011).

In ammonia recycle percolation, the liquid ammonia is percolated through the biomass to disrupt the complex polymer matrix. Compared to other pre-treatment methods, ammonia recycle percolation produced no

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

inhibitor. The general drawback in this method is high energy consumption.

d) Steam Explosion

In this method, the biomass is expose to high temperature steam between 160^0C - 260^0C and high pressure (Agbor et al., 2011), the degradation of hemicellulose into xylose and glucose monomer is cause by acetic acid produced from hemicellulose acetyl groups during the treatment. This pre-treatment is sometime called auto-hydrolysis.

Catalysts like H_2So_4, Co_2 or So_2 are used to improve the efficiency of this process and it has been found to minimmize formation of inhibitor, improve recovery of hemicellulose sugar and efficient hydrolysis. The major drawback of this treatment is the formation of fermentation inhibitors and compounds like lignin carbohydrate matine and the need to wash the hydrolysate (Agbor et al., 2011).

e) Liquid Hot Water Pre-treatment

This method uses liquid water instead of steam at very high pressure of 5mpa and temperature of between 170 – 230 ^{0}C, it is also called hydro-themolysis, and it removes lignin for easy accessibility of enzyme into hemicellulose and cellulose. It is very effective in treating biomass like softwood (Rabemanolontsoa and Saka, 2016). For the effectiveness of this method, pH between 4 -7 is required to avoid sugar degradation and formation of inhibitor. The general low cost of this treatment makes it attractive.

v) Advance Pre-treatment Method

With the evolution of the concept of green engineering which are developed to mitigate negative impact cause by the used of conventional chemicals to the environmental, more robust and environmentally friendly methods are developed, it includes ionic liquids (ILs) and deep eutectic solvent (DES) based pre-treatment methods.

a) Ionic Liquid

Ionic liquid is considered to be environmentally friendly, due to it high thermal and chemical stability, ability to exist as liquid at relatively low temperature, low vapour pressure, non-flammable etc. it is made up of significant quantity of organic cations and little amount of inorganic ammonia which combined to from salt solution with strong electrostatic bonding. The characteristic of the solution can be designed for specific product by adjusting the combination ratio of the components.

Many studies revealed that lignocellulose biomass treated unto lonic liquid shows an increase surface area, reduce percentage of lignin and cellulose depolymerization. (Kassaye et al., 2017). Examples of ILs includes: 1-ethyl-3-methylimidazolium acetate, Cholinium lysine, 1-ethyl-3- methylimidazolium diethyl phosphate, 1-butyl-3- methylimidazolium chloride, Tetrabutylammonium hydroxide, etc.

The major drawback of this pre-treatment method is high cost of ILs chemicals which influences the overall cost of the process.

b) **Deep Eutectic Solvent Pre-Treatment**

Deep eutectic solvent is a tunable or designer solvent prepared from a mixture of organic compounds with hydrogen bond acceptor. For examples, potassium carbonates glycerol, choline chloride, and lactic acid (Lugani et al., 2020). It is a major alternative to ILs, thus it possesses similar properties as ILs.

Over the years, deep eutectic solvent has been used as electrolyte, reaction media, and solvent for extraction and recently as solvent.

For depolymerization of lignocellulose during pre-treatment, a report by Lee, (2019) indicated that lignocellulose treated with DES prevents loss of sugars and also exposes the cellulose structure to enzymatic hydrolysis. The used of DES also found to reduce energy utilization by 28% as compared to acid or alkaline pretreatment (Procentese et al, 2017).

3.3 Enzymatic Hydrolysis or Saccharization

After pre-treatment, is the conversion of the lignocellulossic biomass into pentose and hexose sugars through hydrolysis, this becomes imperative since the enzymes responsible for bioethanol production can digest only monomers. The saccharization process can be catalysed either by an acid or enzymes. The used of acid catalyst for hydrolysis of sugar monomers is to break the hydrogen bond of the cellulose chain and convert the crystallized structure to amorphous form. Both concentrated acid and diluted acid are used for this process. Concentrated acid requireds low temperature condition at short period of time while dilute acid required high temperature condition. Examples of acid predomnasitly used are H_2SO_4 and HCl. However, the

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

disadvantage of using acid catalyst include formation of inhibition agent, corrosive nature of the acid, degradation of the produced sugar, recovery of the acid, disposal, energy requirement of the process etc. (Azhar et al., 2017).

Enzymes hydrolysis on the other hand required mild operating conditions and is environmental friendly without formation of inhibiting agents. The efficiency of enzymes hydrolysis are affected by the following factors; pH, temperature, enzyme loading, time of reaction, substrate concentration and impurity in the reacting vessel. Enzymes used for hydrolysis include modular and non-modular glycosyl hydrolases which are cellulase and hemicellulase, carbohydrate esterases and auxillary activity protein (AA).

a) Glycosyl Hydrolases (GH_ε)

Glycosyl hydrolases initiate the cleavage of glycosedic bond which link two or more sugar or non-sugar unit in oligosaccharide chain. The GH_s group of enzyme comprises of 115 modular and non-modular enzymes of cellulase and hanicellulase. These groups of enzymes catalysed saccharization in two ways, inversion and retention mechanize given rise to products which are steroisomer or identical to the substrate respectively.

There are three types of cellulose enzyme, it includes endoglucanase and exoglucanase, (3 – glucosidases and cellobiohydrolase they catalysed cellulose to produce hexose sugars (Predominantly glucose) (Patel et al., 2019). Endoglucanases act on (3-1, 4 glycoside bonds of amorphous cellulose. Some groups of endoglucanase use a retention mechanism example GH_s families of 5, 7, 12, 44 etc. while some uses inversion mechanism example 6, 9 and 48 GH_s families.

The product of endoglucanase enzymes is mainly oligomer with varying level of polymerization which is subsequently catalyszed by exoglucanases to release cellobiose. The cellobiohydrolases act either as reducing or non-reducing end of cellulose producing short chain oligomers, while (3-glycosidases catalysed the bond cleave of cellobiose and cellodextrin yielding glucose as final product (Patel et al., 2019).

The hemicellulases glycosyl hydrolase enzyme mediates the breakdown of the bond in hemicellulose fraction of lignocellulose. The enzyme involve in this process includes endoxylanase, B-xylosidae, $\propto$ - arabinofiranosidae, acetyle xylan esterase. The major products obtained from the action of these enzymes include short chain xylo-oligomer from xyloboise using B-xylosidae enzyme.

Also, short chain manno-oligonier obtained from degradation of hemicellulose which contained mannanase using B-mannanases and B-mannosidases as enzymes.

Most of these enzymes are very expensive and are required in large amount thus, to reduce the cost of production, microbes with potential of secreting some of these enzymes are used and it includes Clostridium, Bacillus, fuserium, Humicola etc (Imran et al., 2016). Though, microbial enzymes have some short coming like stability, substrate product inhibition and catalyst efficiency.

b) Carbohydrates Esterase

The removal of ester moieties from carbohydrate is mediated by carbohydrates esterases. There are 16 groups of this enzyme ranging from group 1 to 16, though the initial group 10 has been removed from the series due to their activities against non-carbohydrate substrate. The carbohydrate esterases help in initiating the removal of ester-based flag from poly, oligo and monosaccharides. The removal of saccharides flag helps quicken up carbohydrate degradation through easy access of glycosyl hydrolases to the active sites. (Kawaguchi et al., 2017). The most analysed enzymes from this group are acetyle xylan and feruloyl esterases. Feruloyl esterases and acetyle xylan mediate the ester bond holding ferulic acid and acetyle substitution on xylose fractions respectively.

c) Auxiliary Activity (AA) Protein

Auxiliary activity protein is a novel type of lignocellulose degradation enzyme. It is an oxidative enzyme which mediates cleavage of glycosidic bonds in glucose polymers through oxidative pathway. There are nine families of lignin degrading enzyme and 6 females of polysaccharide which constitute auxiliary degradation enzymes and activity enzymes. They assist glucosyl hydrolase and carbohydrate esterases to degraded carbohydrate in lignocellulose. The lignin degradation enzymes from AA protein include AA1, AA2, AA3 and AA8. These are the most studied families of auxailiary activity protein which act on lignocellulose to degradate lignin via oxidative route. (Bodenheimer et al., 2018).

Polysaccharide degradating enzymes of auxiliary activity protein includes AA9, AA10, AA11 and AA13

these are the most important enzymes of this series. Their main source is fungi. The lystic polysaccharide enzymes uses copper ion present in their domain to trigger hydroxylation of C_1 or C_4 in polysacchandes substrates to produce either aldonic acid or 4-keto sugars respectively.

3.4 Lignocellulose Enzymes from Microbial Sources

There are basically two strains of microbes that produced lignocellulotytic enzymes, there are filamentous fungi/aerobic bacteria which produced class of enzymes called extracellular enzymes and cellulosome in anaerobic bacteria and fungal strain (Patel et al., 2019). Some anaerobic microbes include clostridium, piromyces, bacteroides etc. while aerobic microbes include Streptomyces spp, pseudomonas sp. Nocardia sp etc though bacteria are very useful in lignocellulolyta enzymes production, and fungi are the most widely used. Example of such fungi class includes T. reesei, aspergillus sp, P. decumbens, M. thermophile etc

Over the years, strain improvement strategies have been adopted to increase the efficiency of these microbes while reducing the overall cost of production cause by the used of some of those microbes' mentions above. Some of these strategies includes random mutagenesis, site directed mutageneses, heterogenous expression of protein and gnome and metabolic engineering expression (Wen et al., 2020). All these methods have been found to increase the efficiencies of the affected microbes.

3.5 Fermentation Processes

This is a vital step in ethanol production, which involves the conversion of the monomeric sugar obtained in the hydrolysis step to ethanol, acids, and gases through microbial action. Some of the widely used microbes include S. cerevisiae, E. coli, Candida shehatae, Scheffersomyces stipites, Zymomonas mobilis, etc (Lugani et al., 2020). This process is controlled by some factors like pH, temperature of the system, aeration rate, concentration of salt, carbohydrate concentration, and ethanol concentration. The efficiency of the process depends on the microbial strength, the ability of the microbes to withstand high concentrations of substrate, sugar, ethanol, and other by-products. The initial challenges of natural microbes withstanding harsh operating conditions for optimum yield have been overcome by the development of genetically modified microbes. The fermentation process can be done in batch, fed-batch, or continuous mode depending on the capacity of the production process.

i) Methods Adopted for Fermentation

Many approaches have been adopted for the fermentation of sugar monomers to ethanol, such methods include separate hydrolysis and fermentation, simultaneous saccharification and fermentation, simultaneous saccharification and fermentation, filtration and fermentation, consolidated bioprocessing, non-isothermal simultaneous saccharification and fermentation, etc.

a) Separated Hydrolysis and Fermentation

In this method, enzymatic hychrolysis and monomer fermentation are performed in a separated systems, this allow for optimum reaction conditions for both processes. They are separated and subjected to their different optimum reaction condition, this help in maximizing yield obtained though costly and longer reaction time is needed.

b) Consolidated Bioprocessing

In this method, three different processes are performed in the same system, which include enzyme production, conversion of biomass substrate into monomer, and the fermentation process. The commonly used microbe for this process is Clostridium thermocellum reason being that it can easily convert lignocellulose to monomer sugar, which gives way to ethanol production. The process is very efficient, and it minimizes the environmental impact of an extensive process.

c) Simultaneous Saccharification and Co-fermentation (SSCF)

This method includes enzymatic hydrolysis and sugar monomer fermentation being performed in a single system. A modified strain of Saccharomyces cerevisae is used because of its ability to ferment pentose sugar. The only challenge with this method is controlling process parameters for optimum yield since both processes are performed in a single system (Damayanti et al., 2021).

3.6 Ethanol Recovery

This is one of the most important stages in ethanol production. It involves the separation of the ethanol from the broth after the fermentation process. The water

content of the broth is reduced to about 0.5% which raises the concentration of the anhydrous ethanol to about 99.5%. The reduction is profound using processes like simple distillation, azeotrophic distillation. Dehydration, membrane separation, and extractive distillation among these processes, the most used method is extractive distillation (Hajinezhad et al., 2021).

3.7 Life Cycle Analysis (LCA)

Life cycle analysis (LCA) of bioethanol is performed to assess the environmental impact of producing and using bioethanol from different feedstock, right from the start of production till disposal of the final waste. It assesses all environmental impacts like gas emissions, resource depletion, and land used for cultivation. The basic methodology used for LCA includes goal and scope definition, life cycle inventory (LCI), life cycle impact assessment (LCA), and result interpretation.

The usefulness of LCA cannot be overemphasized because it helps in the identification of the potential impact of bioethanol production from different biomass before optimization of the process.

Tools used for LCA include LUCAS, Lune, TrACI, ReCiPe, Ecoindicator 99, and CML 2022. (Rosenbaum et al., 2017). Several LCA performed on bioethanol production and utilization showed that it has substantial potential to replace fossil fuel while also mitigating carbon footprint, which in turn protects the ozone layer from depletion (Edeh, 2021).

4 Biobutanol

Biobutanol is an exceptional alcohol which has a unique property that makes it a promising substitute for conventional gasoline, such properties include but not limited to high combustion heat, low volatility, low hygroscopicity, elevated energy density, less corrosive nature, and above all, the ability to mix with gasoline at a ratio up to 85% without modification of conventional Otto engine cycle. These exceptional characteristics make biobutanol a more valid source of energy than even the conventional gasolines.

Globally, the estimated market value of biobutanol in 2025 stood at 1.18 billion USD, and it is projected to reach 3.54 billion USD by 2034. The projected rapid increase is attributed to novel production methods, which minimize the gross production cost (Ganeshan et al, 2025).

4.1 Biobutanol Production

4.1.1 Microbial Species used for Biobutanol production

The isolation of the Clostridium genus by Weizmann in 1912 marks the beginning of research into various microbes that can ferment biomass for the production of bioethanol. The genus Clostridium comprises over 40 species of Gram-positive anaerobic bacteria which are solventogenie in nature. The microbes are rod-shaped in nature with peritridious flagella that enable them to be mobile, although some environmental factors may influence the shape of the microorganism. Clostridium species undergoes fermentation through two destructive processes of acidogenesis and solvantogenesis. The fermentation process of this kind is sometimes referred to as biphase fermentation. The main products of this fermentation are acetone, biobutanol, and ethanol (ABE); nevertheless, other products like acetic and butyne acids, gases (CO_2 and H_2) are also obtained (Sánchez-Ramírez et al., 2023). The acidogenesis involves the utilization of glucose to generate ATP in the presence of NADH – abundant conditions. The acid produced in this process (acetate and butyrate are reabsorbed as substrate during solventogenesis for the production of ABE in a likely ratio of 6:3:1 (butanol, acetone, ethanol), Diaz et al., 2023). The production of these trio solvents affects the bacterial cell metabolism, and at a certain level of concentration > 13g/l. the metabolic activities in the cell ceased.

Clostridum species also produce biobutanol from two other pathways using different sugars. The pathways are the isopropanol-butanol ethanol pathway (HBE). The major distinction of these pathways is in substrates (sugars) and the species of Clostridium used for the fermentation process (Liberato et al., 2019).

Aside from the solventogenic bacteria, non-solventogenic bacteria also produce butanol following a different pathway. Clostridum species, such as Lactobacillus species, belong to this group. They produced 2-butanol instead of 1-butanol within the bacterial cell wall during anaerobic fermentation of sugar through the reduction of 2, 3, 3-butanediol (Russmayer et al., 2019). Examples of Clostridium Sp used for biobutanol production are C. tyrobutyricum, C. pasteurianum, C. acetobutylicum B18, C. aurantibutyricum, C beijerinckii 8052, C beijerinckii P260, C beijerinckii 592, C beijerinckii BA101, C. saccharobutylicum P262, etc

4.1.2 Feedstock for Biobutanol Production

Just like the bioethanol feedstock, the biobutanol feedstock is classified into four generations, which are the first generation, second generation, third generation, and fourth generation.

i) First-generation Biobutanol Feedstock

The first-generation biobutanol feedstock was made up of edible biomass such as corn, millet, cassava, molasses, sugarcane, rice, etc., from which substrates such as lactose, sucrose, mannose, fructose, glucose, melezitose, xylose, dextrin, trehalose, and galactose were extracted for Clostridium sp solventogenises to produce biobutanol. The main drawbacks of this feedstock were food crises resulting from the use of this biomass and the high cost of raw material (Dehhaghi et al., 2020). The production of butanol from these materials involves soaking the substrate in a mild solution of sodium metasulfite, as a concentrated solution would affect microbes during solventogenesis. The microbes widely used in the first generation were C. acetobutylicum DSM 792.

ii) Second-Generation Feedstock

Lignocellulose and hemicellulose biomass were the major sources of biobutanol in the second generation. The source were municipal residues, forestry agro-raw material etc. this materials eradicated the food cries witness in the first-generation but the production of sugar from these materials was the major challenge as it involves complex physical and chemical process to breakdown the complex structure of the biomass, thus, the conversion of lignocellulose and hemicellulose to butanol require pre-treatment to expose the sugar for easy accessibility of the microbes (clostridium sp) during fermentation. The pre-treatment of this biomass is very necessary because, unlike the first-generation feedstock (Starch), the lignocellulose and hemicellulose, being a complex polymer, contain, aside from the sugar, compounds like acetic acid, furfural, glucuronic, melanoid, phenolic compounds, 5-hydroxymethyl, p-coumaric acid, formate, etc. The pre-treatment involves exposure of the recalcitrant structure for easy accessibility of the microbes, reduction or removal of some of these compounds in order not to inhibit the fermentation process due to their toxicity.

After the pre-treatment, which involves similar processes used for pre-treatment of lignocellulose and hemicellulose for bioethanol production, hydrolysis of the pre-treated biomass is done to obtain fermentable sugars from the feedstocks. The product of the hydrolysis step is subjected to fermentation using the microbes (clostridium sp) to obtained ABE, IBE or HBE depending on the species of clostridium used for the fermentation process. It is worthy to note that the pre-treatment and hydrolysis steps are similar to the processes used for bioethanol mentioned in earlier part of the chapter. Figure 4 shows production pathway of ABE using Clostridium acetobutylicum ATCC 824, the left acidogenesis are indicated in the left handside while the right handside indicated solventogenesis.

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

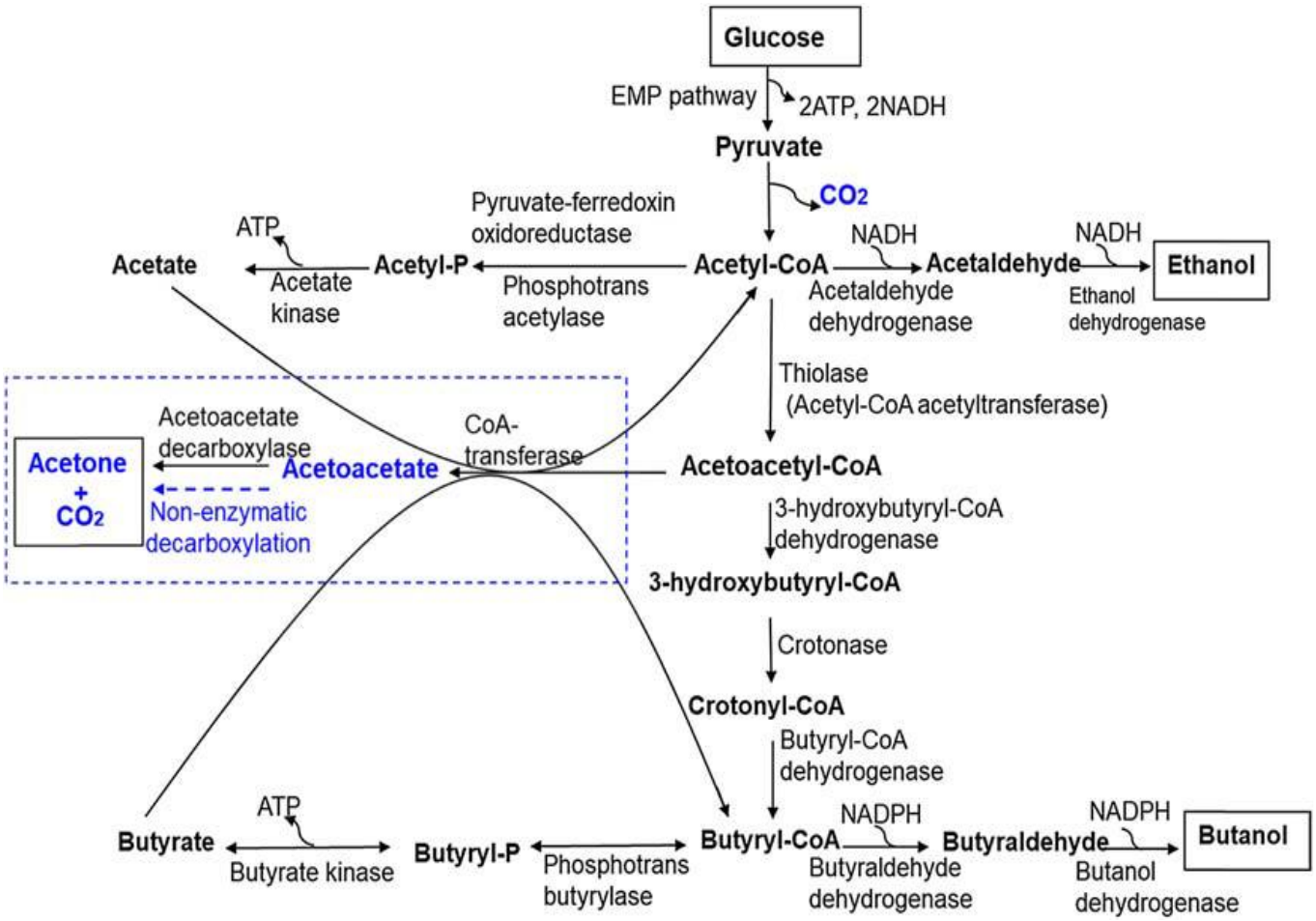


Figure 4: Acetone-Butanol-Ethanol metabolic pathway using C. beijerinckii and C. acetobutylicum. (Han et al, 2011) with permission from Springer. Copyright©

Obtaining the biobutanol from the broth after fermentation through separation and subsequent purification is very expensive as compared to bioethanol due to its complexity and low percentage concentration of biobutanol in the fermented broth. Comparatively, about 2w/w% of biobutanol is present in the broth as compare to 15w/w% of bioethanol in ethanol major challenge is the close boilng point of butanol and water (93 and 100^{0}C) respectively which mitigate the used of ordinary distillation for the purification of the butanol. These factors increases the overall cost of production biobutanol from the second – generation feedstock (Chadni et al., 2023)

iii) Third Generation Feedstock

The third generation feedstock witnessed the used of microalgae as biobutanol feedstock. Microalgae are phytosynthetic microorganism that can grow in fresh water, wastewater, seawater ponds etc. it does not required special land like crops, thus, it posed no food crises. Microalgae are very useful microorganisms due to their usage as animal feed, remediating oil-spilled polluted water, fertilizer, etc. An average of 1.83kg of CO_2 is produced I kilogram of microalgae biomass. There are basically two groups of microalgae, the lipid-rich group, which includes chlorella, Nannochloropsis, Scenedesmus, Butryococcus brauiri, etc, and carbohydrate-rich microalgae, which include dunaliella tertiolecta, phaeodactylum tricornutium, Nitzschia closterium, chlamydomaonas reinharatii, etc. The carbohydrate-rich microalgae contained more than 40% starch, which makes them a good feedstock for biobutanol production. The major advantage of

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

microalgae is that it does not require harsh pre-treatment because of the absence of lignin in its structure, but it contains trace amounts of furfural and 5-hydroxymethyl furfural ($0.5gl^{-1}$) when hydrolyzed with 3% H_2S0_4. Pre-treatment methods used for microalgae include mechanical, thermal, chemical, and biological (enzymatic). The procedures are as described for bioethanol, but under mild conditions.

During the fermentation step, several species of Clostridium can be used depending on the type of sugar present in the feedstock, and also the source of the lignocellulose and hemicellulose.

iv) Fourth Generation Biobutanol Feedstock

Unlike other biofuels, little research has been done on the production of biobutanol from fourth-generation feedstocks, which are genetically modified microorganisms (micro-algae and macro-algae). However, algae species like chlorella, neochloris, and Nannochloropsis have been modified, and the results showed high lipid content with potential for optimization for more biobutanol yield. (Shokravi et al., 2022).

One means through which optimization of yield can be done is by the addition of reducing agents like ascorbic acid, L-cystene etc., to the strain. The reducing agent acts as an electron donor, influencing specific genetic expression, which in turn improves metabolite flux in the direction of the aimed metabolite (Chandgude et al., 2021). The challenge is the construction of some of the algal genes for biobutanol production due to their complexity. Only 3000 species of algae out of over 40,000 species have the potential of being used as biofuel feedstock presently, only about 30 species have been genetically constructed for biobutanol production.

v) Syngas as Biobutanol Feedstock

Synthetic gas (syngas) is a mixture of CO_2, CO, and H_2, which is generated when biomass is gasified. Some species of Clostridium, such as C. carboxidivoraus, C. Butyribacterium, C. ragadalci, and C. Ijungdahlii, absorbed carbon from syngas for metabolism and production of ethanol and butanol. The use of syngas for biobutanol production is promising due to the availability of the feedstock (biomass) and its usage does not pose food-energy crises. The production of biobutanol ethanol and other compounds from synthetic gas utilizes all the carbon components of the lignocellulose but comparatively, the quantity of yield is minimal, this can be attributed to the utilization of most of the energy by Nicotinamide adenine dionucleotide + hydrogen (NADH) in the cause of reduction of CO and CO_2 into biobutanol, ethanol, butyrate etc. which demand more energy than reduction of pyruvate in cellular metabolism. Another factor militating efficient biobutanol production from syngas is the low solubility of H_ε, CO, and CO_2 ($0.0355mgl^{-1}$ and $27mgl^{-1}$ at, $25^{-0}C$ respectively during bioconversion. This led to the suggestion of pressure fermentation of > 2atm to allow H_2, CO_2, and Co to dissolve completely for a more efficient process (Phillip et al., 2025). The availability of impurities like HCN, H_2S, NO, NO_2, ethylene, acetylene, etc, also posed a serious challenge during the fermentation of the substrate.

4. 1. 3 Butanol Fermentation Process

Fermentation converts the monomer sugar using a microorganism (Clostridium sp) into biobutanol. The process occurred within specific operating conditions of temperature, pH, agitation speed, and time. Common batch fermentation takes place between 35 to 37 °C, approximately pH of 5, and between 32 to 72 h, depending on the type of strain used. In a batch fermentation, the substrate, nutrients, and the microbes are charged into the fermenter at the beginning of the process thereafter, incubation at specified conditions is performed for optimum growth of the microbes and subsequent yield of butanol. The fermentation process may be either anaerobic or aerobic, depending on the targeted product(s)

However, aerobic conditions boost up metabolic process in the cell, producing more ATP, thus for bio-butanol production, anaerobic condition is preferred. At the beginning of anaerobic fermentation, Nitrogen or Carbon (iv) dioxide gas is used to maintain the osmotic pressure of the system until the microorganism begins to generate gases to stabilize the pressure of the system. When fermentation is completed, processes like centrifugation, filtration, etc, are used to separate the cell mass from the liquid. The butanol is then obtained via distillation of the broth. A typical ratio of the solvent is 3:6 of acetum butanol and ethanol (ABE) respectively.

i) Continuous Fermentation

Continuous fermentation requires the fermentation process to be continuous for a long time without any downtime using one inoculum. It is essential because it

Academic Journal of Innovative Engineering and Technology
Vol.06, No. 04; April-2026;
ISSN (2343–403X);
p –ISSN 3244–5621
Impact factor: 6.37

reduces the cost and time of operation and produces a high percentage yield if properly managed. There are basically two techniques adopted for continuous fermentation: free cell and immobilized cell recycling techniques.

The free cell fermentation required continuous feeding of nutrients and substrates to the reactor and continuous removal of an equal volume of effluent from the bioreactor. With this approach, a yield of above 14.20g/l^{-1} can be obtained before the acidogenic process set-in.

The immobilized cell recycling technique is an advancement of the free cell technique because the cell can be cultured for a longer time than in the free cell technique, which gives a higher percentage of ABE than the free cell approach. An example of a cell used for this technique is the C. acetobutylium cell in a convoluted fibrous bed bioreactor.

4.2 Techniques used to advance biobutanol fermentation

Due to challenges encountered during the fermentation process, some novel techniques have been applied to improve the process and subsequent improvement of the quality of biobutanol obtained, such processes include co-culture fermentation and the use of electron donors. Co-culture fermentation involves the application of a second strain into the bioreactor using the general idea that bacteria live in a colony and complement each other. The second microbe introduced has a specific task to perform in the fermentation process. The sharing of the task reduces the burden on one bacterium thereby increasing the efficiency of the process.

One of the approaches in this technique is the use of cellulolytic strains, which are responsible for producing monomeric sugars from lignocellulosic biomass. The reducing sugar can then be used for growth by solventogenic bacteria and, in turn, produces the solvents (ABE). Examples of co-culture bacteria are C. thermocellum and C. beijerinckii. Another approach is the use of aerobic bacteria to remove excess oxygen in the bioreactor because of its negative effect on the system. In this approach Bacillus genre is commonly used for the removal of oxygen.

Additional efficiency of the fermentation process can also be obtained by the use of two different strains of solventogenic bacteria. This also boasts the strength of the process with a resulting high yield. Some microbes that secrete amino acids can also be introduced into the bioreactor, because studies revealed that some solventogenic bacteria use amino acids for energy production, which in turn would boost the production process. Examples of such bacteria include Saccharomyces cerevisiae (Wu et al., 2019).

The use of electron acceptors is a new approach used in optimizing biobutanol yield during fermentation. In this approach, a reducing agent is introduced into the broth to enhance the redox potential of the broth toward the aimed metabolite. It influences the generation of a specific gene that improves the yield of biobutanol. The application of this method was adopted by Ding et al (2018) when sodium sulfate was added as an electron receptor to corn meal substrate using C. acetobutylicum as microbes. The result shows a 34.8 % increase in yield.

4.3 Separation of Biobutanol from Broth

The separation and purification of the solvents (ABE) is another challenging aspect of butanol production from biomass. Therefore, the traditional distillation method was used, but due to the high boiling point of butanol (118 ^{o}C), the energy cost of the process was much which in turn increases the overall cost of production. Thus, some improved integrated methods have been proposed, these include pervaporation, gas stripping, liquid-liquid extraction, perstraction, and in-situ extraction – gas stripping.

i) Perstraction

This is an advanced liquid–liquid extraction which involves the use of a selective membrane. The two solutions, the extraction solution and fermentation broth, are on either side of the membrane. This prevents direct contact of the two solutions; this method prevents the harsh or toxic nature of the extractant from contaminating the fermentation medium. Examples of such extractants include oleyl alcohol, 1-dodecanol, 1-octanol, tributyrin, etc. The efficiency of this method depends largely on the type of membrane, the thickness of the membrane, type of extraction used. The drawbacks of this method are the high cost of the membrane and fouling (Solis et al., 2023).

ii) Pervaporation

Pervaporation involves partial vaporization of a multicomponent mixture through, permeable membrane. In this method, only the target liquid passes through the membrane. It is an efficient method of separating liquids

of similar boiling points. The method involved evaporating effluent from the fermenting medium, which contained butanol, contacting it with one side of the membrane, and transferring it as vapour with the aid of sweep gas. The method is based on diffusivity rather than volatility (Wang et al., 2017). The permeated vapours are condensing to recover the butanol. The method did not affect the fermenting medium. Examples of membranes used include silicon, aluminium-free zeolite, etc.

iii) Gas Striping

In this method, fermentation effluent is made to pass through the rotating shaft of the bioreactor by pumping oxygen-free nitrogen through the fermenter. This method helps in separating volatile components of the effluent. The volatile components, which are mainly ABE, are condensed while the stripping gases are recycled into the fermenter. The method is very friendly with microbes as it has no negative effect on their physiology and the fermenting medium. The major constraint of this method is the hyperfoaming nature of the bioreactor at the initial stage, but it can be overcome by the use of an anti-foaming agent like polypropylene glycol (PPG). To improve the efficiency of this process, appropriate conditions in the fermenter, appropriate dosage of anti-foaming agent, and gas flow rate must be carefully selected.

iv) Liquid-Liquid Extraction

In liquid –liquid extraction, two or more immiscible liquids are mixed with the purpose of reducing the toxicity of the fermenting medium and removal of the targeted solvent. The effluents from the broth are moved to the extractant and are separated by simple distillation. The efficiency of the process depends on the choice of the extractant and other operating conditions. Though a very successful integration process, it is very expensive and if not properly managed, it can result in environmental toxicity, corrosion of the equipment, emulsion formation, and loss of essential by-products.
Other integrated separation processes are also applied to optimized biobutanol recovery from the fermenting medium, such processes include gas entrapment membranes, direct steam distillation, wall column distillation, gas tripping – pervaporation, pervaporation – pervaporation, gas stripping – salt in, and pervaporation-salting out.

5 Conclusion

Over the years, the development of biofuel production technologies has evolved with the advancement of new methods that focus on optimization of yield and mitigation of the high cost of production. To achieve this, genetically constructed microbes, an integrated fermentation-separation process, and an integrated separation-separation process are researched for best practices that will facilitate high yield of butanol and bioethanol from biomass. As researchers continue to push boundaries, it is believed that in the near future, the utilization of biobutanol, biodiesel, and bioethanol as alternative energy sources would come to stay and help in solving global energy crises and reduce carbon footprint.

References

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., & Levin, D. B. (2011). Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances, 29*(6), 675–685.

Akpan, I. O., Edeh, I., & Uyigue, L. (2023). A review on biodiesel production. *Petro Chem Indus Intern, 6*(2), 131–141.

Akubude, V. C., & Nwaigwe, K. N. (2016). Economic importance of edible and non-edible almond fruit as bioenergy material: A review. *American Journal of Energy Science, 3*(5), 31–39.

Azhar, S. H. M., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Faik, A. A. M., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports, 10*, 52–61.

Bodenheimer, A. M., O'Dell, W. B., Oliver, R. C., Qian, S., Stanley, C. B., & Meilleur, F. (2018). Structural investigation of cellobiose dehydrogenase IIA: Insights from small-angle scattering into intra- and intermolecular electron transfer mechanisms. *Biochimica et Biophysica*

Acta (BBA) - General Subjects, 1862(4), 1031–1039.

Carrillo-Nieves, D., Rostro Alanís, M. J., de la Cruz Quiroz, R., Ruiz, H. A., Iqbal, H. M. N., & Parra-Saldívar, R. (2019). Current status and future trends of bioethanol production from agro-industrial wastes in Mexico. *Renewable and Sustainable Energy Reviews, 102*, 63–74.

Chadni, M., Moussa, M., Athès, V., Allais, F., & Ioannou, I. (2023). Membrane contactors-assisted liquid–liquid extraction of biomolecules from biorefinery liquid streams. *Separation and Purification Technology, 317*, 123927.

Damayanti, D., Supriyadi, D., Amelia, D., Saputri, D. R., Devi, Y. L. L., Auriyani, W. A., & Wu, H. S. (2021). Conversion of lignocellulose for bioethanol production applied in bio-polyethylene terephthalate. *Polymers, 13*(17), 2886.

Dehhaghi, M., Panahi, H. K. S., Jouzani, G. S., Nallusamy, S., Gupta, V. K., Aghbashlo, M., & Tabatabaei, M. (2020). Anaerobic rumen fungi for biofuel production. In *Fungi in fuel biotechnology* (pp. 149–175). Springer.

Díaz, M. J., Ferrero, P. M., & Moya, M. (2023). Sequential acid/alkali pretreatment for an olive tree pruning biorefinery. *Agronomy, 13*(11), 2682.

Dou, J., Chandgude, V., Vuorinen, T., Bankar, S., Hietala, S., & Lê, H. Q. (2021). Enhancing biobutanol production from biomass willow. *Journal of Cleaner Production, 327*, 129432.

Edeh, I. (2020). Biodiesel production as a renewable resource for the potential displacement of petroleum diesel. In *Biorefinery concepts, energy and products*. IntechOpen.

Edeh, I. (2021). Bioethanol production: An overview. *Bioethanol Technologies, 1*.

Ganeshan, S., & Tülbek, M. Ç. (2025). Fermentative butanol production—Perspectives and scale-up challenges. *Encyclopedia, 5*(2), 50.

Hajinezhad, A., Lak, M., & Alimoradiyan, H. (2021). Optimum energy analysis of separation and purification units for bioethanol production. *International Journal of Energy Research, 45*(10), 15452–15462.

Han, B., Gopalan, V., & Ezeji, T. C. (2011). Acetone production in solventogenic *Clostridium* species. *Applied Microbiology and Biotechnology, 91*(3), 565–576.

Hayyan, A., Alam, M. Z., Mirghani, M. E. S., Kabbashi, N. A., Hakimi, N. I. N. M., Siran, Y. M., & Tahiruddin, S. (2011). Reduction of free fatty acid in sludge palm oil for biodiesel production. *Fuel Processing Technology, 92*(5), 920–924.

Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance digestibility of lignocellulosic biomass. *Bioresource Technology, 100*(1), 10–18.

Imran, M., Anwar, Z., Irshad, M., Asad, M. J., & Ashfaq, H. (2016). Cellulase production from fungi and bacteria. *Advances in Enzyme Research, 4*(2), 44–55.

Kassaye, S., Pant, K. K., & Jain, S. (2017). Hydrolysis of bamboo biomass into sugars. *Renewable Energy, 104*, 177–184.

Kawaguchi, H., Katsuyama, Y., Danyao, D., Kahar, P., Nakamura-Tsuruta, S., Teramura, H., & Wakai, K. (2017). Caffeic acid production by fermentation. *Applied Microbiology and Biotechnology, 101*(13), 5279–5290.

Khaliq, A. D., Chafidz, A., Lukman, M. A., & Kholil, I. (2020). Bioethanol production from banana weevil. In *IOP Conference Series: Materials Science and Engineering* (Vol. 722, p. 012080).

Lee, J. W. (Ed.). (2012). *Advanced biofuels and bioproducts*. Springer.

Lee, J.-W., & Jeffries, T. W. (2011). Efficiencies of acid catalysts in biomass hydrolysis. *Bioresource Technology, 102*(10), 5884–5890.

Ling, H., Teo, W., Chen, B., Leong, S. S. J., & Chang, M. W. (2014). Microbial tolerance engineering. *Current Opinion in Biotechnology, 29*, 99–106.

Lugani, Y., Rai, R., Prabhu, A. A., Maan, P., Hans, M., Kumar, V., Chandel, A. K., & Sengar, R. S. (2020). Advances in bioethanol production.

Mori, T. (2015). Preparation and characterization of lignocellulose.

Nogales-Delgado, S. (2025). Biodiesel production and life cycle assessment. *Energies, 18*(13), 3338.

Patel, S. K. S., Gupta, R. K., Das, D., Lee, J.-K., & Kalia, V. C. (2021). Continuous biohydrogen production. *Journal of Cleaner Production, 287*, 125037.

Phillips, P. J., Hahn, C. A., Fontana, P. C., Yates, A. N., Greene, K., Broniatowski, D. A., & Przybocki, M. A. (2021). Four principles of explainable artificial intelligence.

Procentese, A., Raganati, F., Olivieri, G., Russo, M. E., Rehmann, L., & Marzocchella, A. (2017). Low-energy biomass pretreatment. *Bioresource Technology, 243*, 464–473.

Rabemanolontsoa, H., & Saka, S. (2016). Pretreatments of lignocellulosics. *Bioresource Technology, 199*, 83–91.

Ramos, M., Dias, A. P. S., Puna, J. F., Gomes, J., & Bordado, J. C. (2019). Biodiesel production processes. *Energies, 12*(23), 4408.

Rathnam, V. M., Modak, J. M., & Madras, G. (2020). Non-catalytic transesterification of microalgae. *Fuel, 275*, 117998.

Rosenbaum, R. K., Hauschild, M. Z., Boulay, A.-M., Fantke, P., Laurent, A., Núñez, M., & Vieira, M. (2017). Life cycle impact assessment. In *Life cycle assessment: Theory and practice* (pp. 167–270). Springer.

Russmayer, H., Marx, H., & Sauer, M. (2019). Microbial 2-butanol production. *Biotechnology for Biofuels, 12*, 262.

Sánchez-Ramírez, E., Huerta-Rosas, B., Segovia-Hernandez, J. G., & Quiroz-Ramírez, J. J. (2023). Biobutanol industrial production.

Shokravi, H., Heidarrezaei, M., Shokravi, Z., Ong, H. C., Lau, W. J., Din, M. F. M., & Ismail,

A. F. (2022). Fourth-generation biofuel. *Journal of Biotechnology, 360*, 23–36.

Solis, C. M., San Juan, J. L., Uy, J. G., & San Juan, J. L. (2024). Optimization of algal biofuel production. *Clean Technologies and Environmental Policy, 26*(4), 1107–1133.

Tabatabaei, M., Aghbashlo, M., Dehhaghi, M., Panahi, H. K. S., Mollahosseini, A., Hosseini, M., & Soufiyan, M. M. (2019). Reactor technologies for biodiesel production. *Progress in Energy and Combustion Science, 74*, 239–303.

Tayyab, M., Noman, A., Islam, W., Waheed, S., Arafat, Y., Ali, F., Zaynab, M., Lin, S., Zhang, H., & Lin, W. (2018). Bioethanol production from lignocellulosic biomass. *Applied Ecology and Environmental Research, 16*(1).

Wang, Y., Ho, S.-H., Yen, H.-W., Nagarajan, D., Ren, N.-Q., Li, S., Hu, Z., Lee, D.-J., Kondo, A., & Chang, J.-S. (2017). Advances in biobutanol production. *Biotechnology Advances, 35*(8), 1049–1059.

Wen, P., Zhang, T., Xu, Y., & Zhang, J. (2020). Co-production of sugars from poplar. *Industrial Crops and Products, 152*, 112500.

Wu, W., Lei, Y.-C., & Chang, J.-S. (2019). Life cycle assessment of microalgae biofuel. *Bioresource Technology, 288*, 121492.

Yaqoob, H., Teoh, Y. H., Jamil, M. A., Rasheed, T., & Sher, F. (2020). Tribological behavior of pyrolysis oil blends. *Sustainability, 12*(23), 9975.