



Review

New eco-friendly trends to produce biofuel and bioenergy from microorganisms: An updated review

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ABSTRACT

It is critical to ensure the safe disposal of organic residues, especially because the accumulation of organic wastes contributes to environmental contamination; spread of diseases, unpleasant odors; and the release of ammonia and other dangerous gases in the environment. Consequently, researchers are considering various direct organic waste applications, including biotechnological applications with ecological and economical benefits such as the limitation of fossil fuel usage, lowering harmful emissions, boosting the synthesis of cost-effective raw materials, and establishing a suitable platform for a diversity of microorganisms. Biotechnology has produced sustainable bioenergy (biogas, biodiesel, bioethanol, and biobutanol), which is an appealing solution for the disposal of organic materials. Carbohydrates are the main component of the organic fraction, and the bulk of these polymers are easily degradable by microorganisms. Taking random samples from soils exposed to organic wastes, purifying the microbial isolates, and evaluating the microbes' capabilities to identify the most useful strain are all part of the isolation process. As a result, this current review focuses on isolated strains of various microorganisms that may use one or more types of organic wastes as the sole carbon source, and to manufacture biofuel as a product from various residues.

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1. Introduction

Due to the global rise in energy consumption and the recent increase in global oil prices, biofuel sectors are garnering renewed interest. Crude oil is currently the primary source of energy, which is deemed unsustainable and global energy consumption is expected to increase by 57% by 2030 (U.S. Energy Information Administration, 2010; Schiffer et al., 2018).

There has been an increase in the environmental impact of petroleum (crude oil) as the sole source of energy, in addition to the concerns about sustainability and economics. Crude oil combustion leads to an increase in the amount of harmful greenhouse

gas (GHG) emissions in the atmosphere, adding to air pollution (Intergovernmental Panel on Climate Change, 2007). As a result, environmental concerns and the rising price of crude oil have fueled research into alternative energy sources. Bioenergy technologies, which include the use of biomass by microorganisms, are one solution. However, much work needs to be conducted to achieve the aim of changing the current economic paradigm into a bio-based economy. The utilization of microorganisms to manufacture various types of biofuels (i.e., alcohols, hydrogen, biodiesel, and biogas) from diverse crude materials, such as carbohydrates, oil crops, agricultural, and animal leftovers, and lignocellulosic biomass is currently being explored (Intergovernmental Panel on Climate Change, 2007).

A biofuel is a form of fuel that acquires its energy from biological carbon dioxide (CO₂) fixation (Demirbas, 2008; Basso et al., 2018). Biofuels are fuels that are produced from live plants, animals, or by-products that are < 20 years old. Biofuels are renewable and create solar energy that has been stored. Plants are generally referred to as renewable energy sources since they may be grown again. Green fuels (biofuels), unlike petroleum-based products, are both biodegradable and environmentally friendly (Abid et al.,

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2016; Weldekidan et al., 2018). While fossil fuels have a long history of carbon fixation, they are not considered biofuels since they include carbon that is no longer in the carbon cycle. Biofuels can be biomass conversion products, solid biomass, liquid fuels, or biogases, among others. Agrofuels, as they are also known, are fuels derived from agricultural products (Weldekidan et al., 2018; Arroyo and Miguel, 2020). Substrates and sources for biofuel production are illustrated in Fig. 1.

Biomass and biowastes are both potential alternatives. Strong biomass biofuels are the most extensively utilized biofuels (Owusu et al., 2016; Boonchuay et al., 2018; Arroyo and Miguel, 2020). As a result, biobutanol, bioethanol, and biodiesel businesses (together with other established biofuels) are projected to rely on procedures with low CO₂ emissions and the use of organic wastes other than those typically linked with the food supply chain, in the future. Dr James Hansen of the National Aeronautics and Space Administration was the first to convey the concept of global warming to the public's notice when he spoke before the United States (US) Senate on the subject in July 1988 (McCarthy, 2012; Ali and Erenstein, 2017).

Fuel production accounts for a major share of the total output. Because they are geared at creating fuels appropriate for transportation and energy production, these are the most crucial fuels to target in attempts to minimize carbon emissions (Hertel et al., 2009, Huang et al., 2015). A surge of interest in renewable energy sources, such as microbiologically generated biofuels, bioalcohols, methane, and hydrogen, has made this possible (Bolzonella et al., 2018; Boock et al., 2019; Adegboye et al., 2021; Malode et al., 2021), for example, stone, charcoal, sawdust, dry manure, and dry grass. This is especially true in poor countries, wherein biofuels are predominantly used to generate heat and account for 70%–90% of the primary energy supply in Africa's least developed nations (United Nations Department of Economic and Social Affairs, 2007).

The microbial fermentation of starch and sugars produces bioethanol, biobutanol, biomethanol, biodiesel, and other liquid biofuels, whereas the transesterification of vegetable oils produces biodiesel (Shereena and Thangaraj, 2009; Selaimia et al., 2015; Lu et al., 2017). Before the discovery of a cheaper supply of fossil oil,

microbial fermentation was employed in a variety of ways, as solvents, greases, cleansers, and important compounds, for the growing chemical industry. Bioethanol and biodiesel are the most commonly used biofuels in transportation, whereas biogas has a small number of large-scale applications in several European areas. Due to features, such as high energy levels, low volatility, and hygroscopicity, biobutanol has recently been regarded as a viable liquid biofuel (Dürre, 2007).

At the same time, it can be used as straight or blended with gasoline without requiring any engine modifications (Pfromm et al., 2010). These types of fuels are classified as second- or third-generation biofuels and defined by the conversion technology that is currently being researched and improved as well as are at pilot or demonstration phases (Balan et al., 2013). Stability, protection, energy density, and predictable combustion are chemical and physical attributes that are required for stable and efficient engine applications. Biodiesel is a liquid biofuel that may be used in diesel engines to replace petroleum diesel (Degfie et al., 2019; Sarno and Iuliano, 2019; Wang et al., 2019).

Extracting oils from vegetable oil feedstock is the most prevalent method for generating biodiesel (Branduardi et al., 2013). Fatty acid methyl esters and fatty acid ethyl esters are the end products of this procedure (Ma and Hanna, 1999). Hydrogen, methane, and CO₂ are among the biogases produced by the anaerobic digestion or fermentation of biomass.

The current review explores the history of biofuels, the current state of microbial fermentation for biofuel production, and some interesting research areas for microbial biofuel production in the future. Furthermore, it examines how the use of microorganism-produced alternative fuels in the past has influenced contemporary biofuel use as well as what the future holds for microbial biofuel development and use.

2. Classification of biofuels

Biofuels are classified according to generations. Conventional biofuels, frequently known as first-generation biofuels, are biofuels

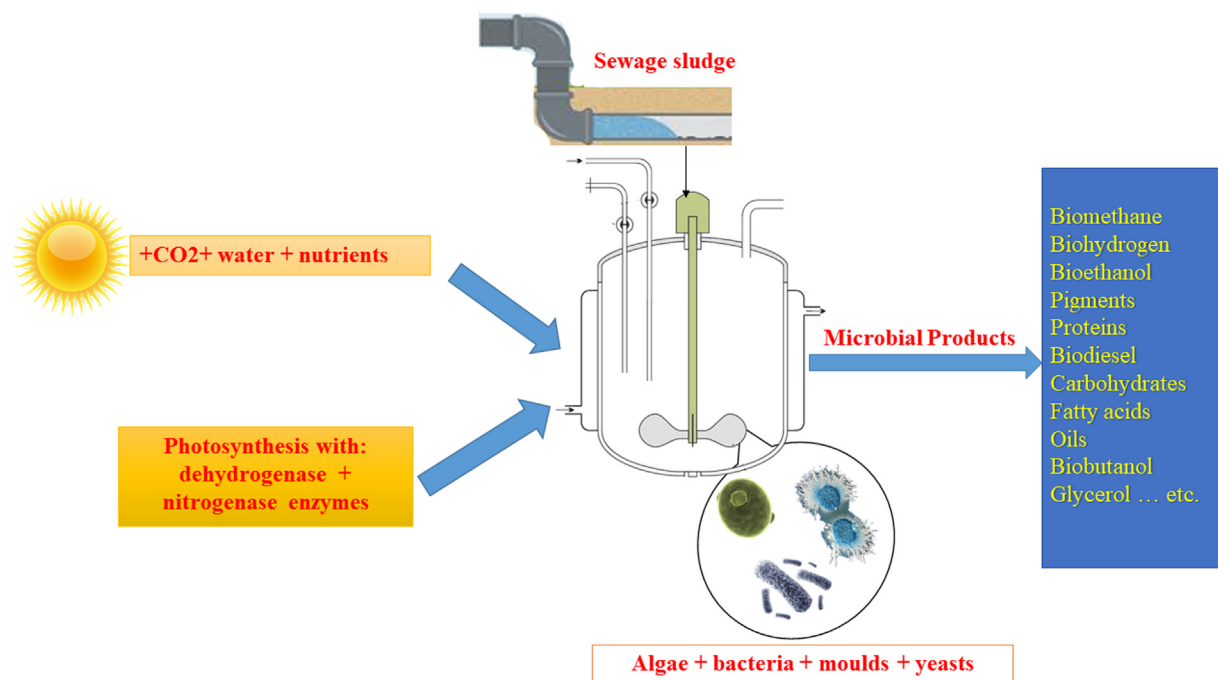


Fig. 1. Substrate and sources for biofuel production.

produced by microorganisms or their enzymes from sugar, starch, or vegetable oil. *Saccharomyces cerevisiae* is the most widely used organism (Arous et al., 2017; Phukan et al., 2019). The synthesis of bioethanol or butanol from starch (wheat, corn, barley, and potato) or sugar (sugarcane, sugar beet, etc.) has been criticized for driving up global food prices (Al-Azzawi and Jassem, 2016; Amoozegar et al., 2019; Corral-Gómez et al., 2021).

The increased pressure on arable land now used for food production could result in severe food shortages, particularly in developing countries, wherein over 800 million individuals are already hungry or malnourished (FAO, 2017). Furthermore, extensive land use, including excessive fertilizer and pesticide applications and water use will have serious environmental consequences (Schenk et al., 2008). Second-generation biofuels are made from renewable feedstock, such as lignocellulosic biomass (Al-Azzawi and Jassem, 2016; Zhu et al., 2020; Carmona-Cabello et al., 2021).

The availability of a feedstock, impact on GHG emissions, biodiversity, and land use are factors in evaluating the long-term viability. Khandaker et al. (2020) produced bioethanol and biodiesel using traditional methods, although with unique starch, oil, and sugar crops, such as *Jatropha curcas*, cassava, or miscanthus (silvergrass). Nonfood plants that do not compete with the production of food lignocellulose (typically 40%–50% cellulose, 25%–35% hemicellulose, and 15%–20% lignin) are the primary component of the plant cell wall and are consequently highly resistant to degradation in biomass (Gray et al., 2006; Schubert, 2006; Cabrol et al., 2017).

Second-generation biofuels are prohibitively expensive to be manufactured in large quantities. Cellulosic ethanol, algal fuel, biohydrogen (H₂), biomethanol, Fischer–Tropsch diesel, H₂ diesel, mixed alcohols, wood diesel, *J. curcas* oil and biodiesel, and others are among the second-generation biofuels being developed (Trabelsi et al., 2018). Algae-based biofuels are the third generation of biofuels. Biodiesel manufactured from microalgae, bioethanol manufactured from microalgae and macroalgae (seaweeds), and hydrogen manufactured from green microalgae and microorganisms are just a few examples of algae-based biofuels (Dragone et al., 2010).

Fourth-generation biofuels are made utilizing petroleum-type hydro-refining, advanced biochemistry, or unique technologies, such as Joule's "solar-to-fuel" system, which does not fit into any other biofuel category. Several of these procedures, however, are either theoretical or limited to the laboratory (Demirbas, 2011; Milledge and Heaven, 2013).

3. Production of biofuels

3.1. Substrate for biogas production

Biogas yield is influenced by a variety of parameters, including substrate type and composition, temperature, moisture, bioreactor design, and microbial composition. However, depending on the type of substrate used, biogas can have a wide range of results. Biogas has been produced using a variety of substrates, including plant and animal wastes, and industrial wastes such as brewery wastes and carbonated soft drink sludge (Suhartini et al., 2021).

Biogas can also be created from a range of sources, including rice straw, municipal solid wastes, wastes dairy manure, chicken wastes, and food wastes, according to several researchers (Khalid et al., 2011; Marañón et al., 2012; Zhang et al., 2013; Fernández-Rodríguez et al., 2014; Gu et al., 2014; Nualsri et al., 2016). Table 1 presents a complete overview of the biogas production capacity of various substrates, indicating that biogas may be efficiently produced from a wide range of organic and waste resources. Overall, the capacity of the anaerobic digestion process to produce biogas, which can be used as a long-term source of heat and power, is

Table 1

A comprehensive review of the potential of different substrates for biogas production.

Substrate	Temperature (°C)	Digestion Time	References
Food Waste	37	225	Scano et al., 2014
Goat manure	35	55	Marañón et al., 2012
Rice straw	37	40	Zhang et al., 2013
Municipal solid	35	200	Song et al., 2014
Fruit wastes and vegetable wastes	35	70	Cheng and Zhong, 2014
Fruit wastes and dairy manure	36	160	Fernández-Rodríguez et al., 2014
Corn straw	37	35	Nagao et al., 2012
Wheat straw	35	45	Gu et al., 2014
Poultry manure	55	20	Nagao et al., 2012
Waste activated sludge	37	10	Zhang et al., 2013
Asparagus stem	35	60	Abouelenien et al., 2010

undisputed. However, further research is required to expand the potential for biogas application and commercialization. Isolating novel bacterial strains capable of producing methane in severe environments could unlock even more potential. Field studies are also required to optimize the factors impacting anaerobic digestion to achieve maximal substrate conversion into biogas (Nualsri et al., 2016).

3.2. Production methods

Methods of production of biofuel are illustrated in Fig. 2.

3.2.1. Direct combustion

This is the most common and oldest type of conversion, wherein organic matter is burned in an oxygen-rich environment only for the purpose of producing heat. Some of its applications include cooking and heating biomass, such as wood, dung, and agricultural wastes in houses, and the burning of wood for fuel in chemical plants (Asadi et al., 2020).

3.2.2. Thermochemical conversion

In contrast to direct combustion, this method produces "synthesis gas" by combining heat and pressure in an oxygen-deficient environment. Synthesis gas is mostly composed of carbon monoxide and hydrogen, and it can be used to generate heat or converted into other fuels, such as ethanol and hydrogen (Williams et al., 2018; Lee et al., 2019; Zhang and Zhang, 2019).

3.2.3. Liquefaction and pyrolysis

Liquefaction and pyrolysis of biomass are two methods for converting products into biomass. The thermal chemical liquefaction process occurs at a low temperature and high pressure in the presence of hydrogen. Hydrothermal liquefaction (HTL) uses subcritical water (SCW) at temperatures ranging from 250 °C to 370 °C and pressures ranging from 40 to 220 bar. Decomposition and polymerization processes that dissolved aqueous chemicals, solid sediments, and gas are all included in HTL. Pressure keeps water in a liquid state, whereas high pressure combined with heat lowers the dielectric constant and density, allowing hydrocarbons to dissolve in water (Dimitriadis and Bezergianni, 2017). The HTL is used for high-water-content biomass and the wood-waste-algae-based biomass as an example is suitable for bio-oil production.

Approximately 700 million dry tons of feedstock and other biomass are produced annually in the USA for biofuel production. According to Langholtz et al. (2016) forest leftovers account for 50% of the feedstock used each year. Residues are appropriate for the synthesis of bio-oil. Wood is the starting material for the HTL

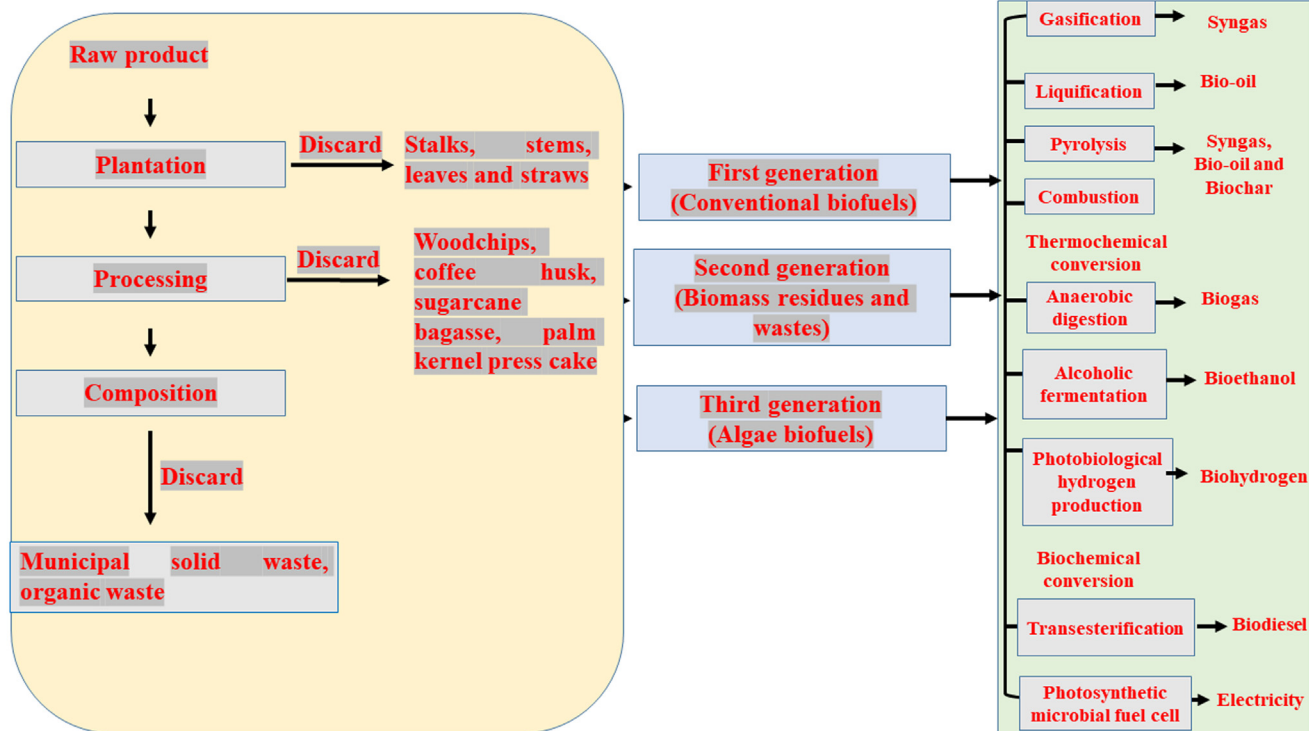


Fig. 2. Methods of production of biofuel.

process, since it includes 50% cellulose, 35% hemicellulose, and 35% lignin. Cellulose dissolves in water at a high temperature and has a high degree of polymerization as well as strong intermolecular interactions and hydrogen bonds (Kumar and Gupta, 2008).

Hemicellulose has fewer hydrogen bonds than cellulose, making it more easily degraded. The amount of oil produced from woody resources using HTL varies depending on the solvent used and the manner of operation, ranging from 17% to 68% (Dimitriadis and Bezergianni, 2017). Deep eutectic solvents are employed as a catalyst in HTL for deoiled *J. curcas* cake by Alhassan et al. (2016). These solvents are simple to make along with low in toxicity, cost, and temperature, with a 41%–54% oil yield.

Sewage sludge is a mixture of lipids, proteins, fibers, nonfibrous carbohydrates, and ash that results from wastewater treatment. The widespread availability of sludge is an excellent starting point for HTL. It was discovered that moist sludge consumes 30% less energy than dry sludge. Several methods are used to lower the moisture content of the sludge, such as utilizing dry straw, flushing, or hexane to remove bound water, methanol to extract the polymer, and pretreatment SCW to break up the sludge cells, resulting in water disposal (Li et al., 2015; Qian et al., 2017; Wu et al., 2017; Biller et al., 2018; Sun et al., 2018).

It was reported that a considerable volume of bound water is released, resulting in a 48% increase in bio-oil production (Yu et al., 2011). The surface tension of the fatty alcohol polyoxymethylene ether AEO9 - SCW was consequently reduced, thereby boosting the hydrocarbon content of the bio-oil and increasing its calorific value by 15%. Not all feedstock are converted to bio-oil in the HTL process; however, there is a nutrient-rich organic matter left in the liquid after plant wastewater (PWW) (Yu et al., 2011). The remaining carbon in PWW is in the form of sugars and organic acids, and technology must be developed to recover the remaining carbon to increase the energy of the resultant oil to 70% (Barreiro et al., 2015; Lu et al., 2017; Li et al., 2019). In the HTL process, wet algae was employed to remove

moisture from sludge and convert lipids, carbohydrates, and proteins into a bio-oil liquid (Wang et al., 2021).

3.2.4. Biochemical conversion

Biochemical conversion, in contrast to direct combustion and thermochemical conversion, occurs at lower temperatures and has slower reaction rates. Fermentation is a natural biochemical conversion process that uses bacteria, yeasts, and other microorganisms to convert sugars and starch from crops, such as sugarcane, corn, wheat, and other grains, into ethanol (Deeba et al., 2017; Hossain, 2019). The method is frequently followed by acid or enzymatic hydrolysis of the cellulosic feedstock to liberate additional sugars from the lignocellulosic biomass (Dhyani and Bhaskar, 2018).

Anaerobic digestion is a biological process that creates biogas in the absence of oxygen through the bacterial degradation of biodegradable organic matter. The chemical transesterification of vegetable oils and animal fats is the most frequent method for producing biodiesel. In various parts of the world, waste cooking oil and fats constitute a major source of pollution. By correctly utilizing and controlling waste cooking oil as a raw material for biodiesel synthesis, this environmental concern could be alleviated. Direct use and mixing, microemulsions, thermal cracking (pyrolysis), and transesterification are the four basic methods for producing biodiesel (Shalaby, 2011; Bridgwater, 2012; Jahirul et al., 2012). Methods of production of biodiesel from glycerol are illustrated in Fig. 3.

The development of microbial fermentation techniques for biodiesel synthesis is gaining favor, since it will allow for the use of a wider range of raw materials. Sugarcane, corn, and biomass are just a few examples. Direct biodiesel production from redesigned cell factories can be divided into two categories: 1) indirect biodiesel production from oleaginous microorganisms via *in vitro* transesterification and 2) direct biodiesel production from redesigned cell factories (Fatima et al., 2016).

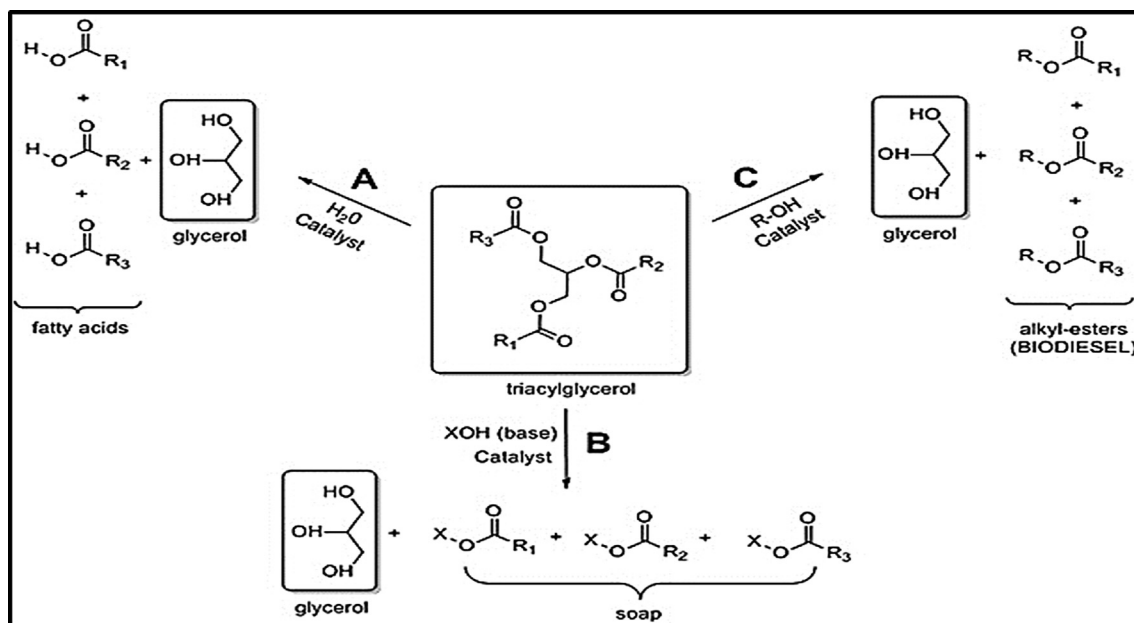


Fig. 3. Methods of production of biodiesel from glycerol.

3.2.5. First-generation biofuels

The price of crude oil has recently fluctuated, prompting several studies towards alternative and supportive energy sources. Biofuels can compete with the rising price of oil to at least offer vehicle fuel. This has resulted in a surge in biofuel research worldwide. The following are the four categories of biofuels: biodiesel (the first commercially available biofuel), ethanol, biogas, and, more recently, biobutanol. These products have been mass-produced in vast quantities and have become an integral part of the manufacturing processes of several countries.

The transesterification of animal fats, plants, and other leftover oils produces biodiesel. This has led to simple engine modifications that allow them to run on biodiesel. However, a complete alternative for ethanol and gasoline may be used in a variety of cars, and it may also be utilized as a raw material to make three-butyl ethyl ether, which is easy to blend with gasoline. Three-ethyl butyl ether is now made from bioethanol. With certain changes, biogas and biomethane are also used in gasoline as a biofuel for cars. They are produced through biological processing, which includes the liquid manure and other harvested raw material methods (Woertz et al., 2009).

The potential to manufacture bioethanol, biodiesel, and biogas from crops that are also used for human nourishment was recently discovered. When it comes to biofuel production, however, crops developed to produce edible oils confront several challenges. There are a variety of multipurpose oily crops (i.e., seeds) that can be used in the industrial biofuel manufacturing process (Woertz et al., 2009). However, in India, the manufacture of biodiesel from *J. curcas*, a perennial shrub or small tree that grows to a height of up to 6 m and produces a sort of vegetable oil blended with biofuel, has received considerable attention. *J. curcas* oil is a low-cost, environmentally friendly substrate that could be used to generate energy from nonedible plant resources. Other solid wastes, such as lignocellulosic wastes, can also be utilized to produce biomaterials, including lignocellulosic waste, which can be used in the second-generation biofuel sector (Woertz et al., 2009).

3.2.6. Manufacturing industry for producing first-generation biofuels

3.2.6.1. Transesterification. Transesterification is a chemical reaction that converts vegetable oils into fatty acid methyl or ethyl

esters, which are then used to make biodiesel. To date, it is the most prevalent method of producing biodiesel. Biodiesel can be used as a substitute in diesel engines because it is physically and chemically comparable to petro-diesel (Demirbas, 2008; Chew et al., 2018).

The esterification of fatty acids and vegetable oils is required to produce economically viable biodiesel, one of the most essential biofuels that are also environmentally friendly. Biodiesel is a renewable energy source made from a variety of natural sources by biochemical reactions using alcohol and a homogeneous or heterogeneous catalyst. As a result of the reaction, a mixture of methyl esters is formed; with a high biodiesel and glycerin content (Kulkarni et al., 2006).

3.2.6.2. Homogeneous catalysis. As illustrated in Fig. 2, homogenous catalysis is a preferable processing method during the slower reaction of its transesterification (base or acid) counterpart. This is accomplished by mixing the reactants and replacing the alcohol from the ester with another alcohol using the same procedure as hydrolysis, except instead of water, alcohol is utilized (Kulkarni et al., 2006).

3.2.6.3. Heterogeneous catalysis. Owing to the interactions of saponification, turning the high oil content of free fatty acids into methyl ester is challenging. It is consequently more efficient to use acidic solid catalysts, which play a significant part in triglyceride transesterification reactions while also converting free fatty acids into methyl ester utilizing Lewis's acid.

On the surface of the catalysts, the esterification of free fatty acids (RCOOH) and methanol (CH₃OH) occurs, whereas the esterification of triglycerides (RCOOR) and methanol is absorbed in the acidic sites (+L). A water molecule can remove tetrahedral during the esterification process, resulting in one mole of ester RCOOCH₃. For bilateral and trilateral triglycerides, it can also be utilized as an esterification process. Esterification is a well-known progressive sequential event, wherein triglycerides are converted to mono-triglycerides and glycerol (Kulkarni et al., 2006).

4. The history of microbial biofuels

Before the industrial revolution, several manufacturing activities were small scale, and the transportation of goods and people was limited. Due to their ability to absorb or trap some of the energy radiated back into space from the heat of the Earth's surface during the industrial revolution in the 19th century, manufacturing processes powered by coal combustion resulted in the production and accumulation of gases collectively known as GHGs, thereby increasing atmospheric temperatures (Khesghi et al., 2004; Boden et al., 2017).

Even though these gases were present in the atmosphere prior to industrialization, the burning of fossil fuels, such as oil, natural gas, and coal, has increased CO₂, methane, and nitrous oxide concentrations by approximately 30%, more than doubled, and approximately 15%, respectively (Martínez et al., 2005). Smoke and smog (a mixture of smoke and fog) became common in London and other industrial centers. This forced the search for alternative energy sources. S. Casey received the first USA patent for using alcohol as a light fuel in 1834.

In 1857, Louis Pasteur discovered lactic acid fermentation and in 1862, Pasteur discovered that certain bacteria may produce butanol, paving the path for the application of microbial fermentation in the production of biofuels. In the 1860s, Nikolaus August Otto was credited as being the first person to use bioethanol in transportation (Antoni et al., 2007; Gottumukkala et al., 2013). Between the 1900s and 1930s, the most significant industrial fermentation in the world was the development of the fuels, ethanol and butanol. Albert Fitz, Martinus Beijerinck, and Sergei Winogradsky discovered related species termed "*Granulobacter saccharobutyricum*," "*Amylobacter butylicus*," and "*Bacillus orthobutylicus*" in their studies on butanol fermentation (Dürre and Bahl, 1996). Nearly four decades after Otto's discovery, Dr. Rudolf Diesel patented a proposal for a compression ignition engine that used peanut oil as fuel.

This vegetable oil fuel supplanted steam engines for a period until the 1920s when diesel engine makers switched to the considerably less viscous petro-diesel, which was both abundant and inexpensive. In 1898, James Dewar succeeded in liquefying chemically produced hydrogen. One-third of Deutz Gas Engine Works' big locomotives were converted to run on pure ethanol in 1902. Owing to the escalating production costs, ethanol was first introduced to gasoline as an anti-knocking addition in 1925. By the 1940s, the price of gasoline had dropped to the point that ethanol had become comparably very expensive, and it had been phased out (Gottumukkala et al., 2013). Increasingly sophisticated fuel injection systems were developed to run these fossil fuel-derived oils (Lan et al., 2013).

The use of fossil fuels has increased over time, resulting in several air pollution disasters, such as the great smog of London in 1952, which killed tens of thousands of people (Pandey et al., 2019). This underlined the significance of altering one's fuel consumption habits. The oil embargo imposed by the Organization of Arab petroleum exporting companies and the Iranian revolution in the 1970s caused the global price of crude oil to skyrocket. As a result, microbial biofuel development has resurfaced in some parts of the world, such as Brazil, wherein a substantial bioethanol sector has emerged based on *S. cerevisiae* fermentation of sugarcane. With 13.7 billion liters produced by 1997 (Basso et al., 2008), Brazil became the world's largest producer of bioethanol (Mabee et al., 2004).

Hydrogen technology was also advanced; in 1988, the first hydrogen-powered aircraft was developed in the USA. The American Clean Air Act of 1956 and 1968 was revised to incorporate more stringent car emission limitations, spurring interest in cleaner and safer fuels. Furthermore, since the late 1990s, crude oil

prices have considerably risen; by 2006, the USA has quickly overtaken Brazil's bioethanol production capacity, with an annual capacity of 18.4 billion liters. In the same year, Nigeria launched a prototype phase of its ethanol project, which focused on the fermentation of sugarcane grown on 10,000 ha in the north. The initiative, however, came to a halt (Ben-Iwo et al., 2016).

David Ramey successfully traveled the USA in a car exclusively driven by butanol in 2005, demonstrating considerable reductions in CO₂, hydrocarbon, and nitrogen oxide emissions (Dürre, 2007). The following year, BP and DuPont began producing fermentative biobutanol from sugar beet. For several years, the anaerobic digestion of organic molecules in industrial wastewater has been used to generate methane, and it is now widely used all over the world (Dev et al., 2019). Several bacteria, including fermentative organisms (acidogens), hydrogen-generating organisms, and acetate-forming organisms, convert polymeric components, such as lipids, carbohydrates, and proteins, to methane and CO₂ in anaerobic conditions (Chubukov et al., 2016).

Acidogens (fermentative microorganisms), acetogens (hydrogen-generating, acetate-forming microorganisms), and methanogens (methane-producing microorganisms) all convert polymeric materials, including lipids, carbohydrates, and proteins, to methane and CO₂ in anaerobic environments (Dornau et al., 2020).

5. Microbial biofuels

The rise in the prices of fossil fuels became considerably steeper early in the third millennium, and biofuels began to acquire popularity as a result of the growing worldwide awareness of pollution and global warming as well as the desire for self-sufficiency (Hwang et al., 2016). In 2011, bioethanol output reached a new high, with the USA leading the pack. Biofuels have gained widespread government support, particularly in industrialized countries, such as the USA and the European Union (EU) (Kot et al., 2016). This has provided the businesses of these countries the economic security they require to invest in various biofuels.

In 2011, South Africa established legislation requiring a mixture of 2% v/v bioethanol with gasoline and 5% v/v biodiesel with diesel, providing the necessary push for biofuel investment and research in the region (OECD-FAO, 2011). In 2010, the global bioethanol consumption was 100 billion liters, whereas the biodiesel consumption was 20 billion liters (OECD-FAO, 2011), and both of these amounts are predicted to continue to steadily rise over the next 10 years. H₂ gas is a less widely used biofuel that is a renewable fuel with a high energy content per unit weight (122 KJ g⁻¹) and produces no particles or greenhouse emissions when burned. Water electrolysis mediated by nuclear or fossil fuels, coal gasification, and natural gas steam reformation as well as biological processes, can all produce hydrogen. Fermentative biological processes for H₂ production are perhaps the most intriguing of these strategies because they consume less energy and can potentially employ trash or agricultural waste streams as raw material. Hydrogenases are hydrogen-evolving enzymes that are required for biological H₂ production (OECD-FAO, 2011).

The reversible oxidation of hydrogen gas is catalyzed by these enzymes (Das and Veziroğlu, 2001; Lynd et al., 2009; Chaubey et al., 2013). According to Collet et al. (2004), lactose fermentation by *Clostridium thermolacticum* provides a low-cost alternative to polluting waste-based H₂ generation. If correctly harnessed, hydrogenases and/or hydrogenase-containing organisms could offer cheap and sustainable H₂ for use as a biofuel or in hydrogen fuel cells to generate power. The current output is limited by the cost of photochemical synthesis reactors as well as low yields (Collet et al., 2014).

6. Microbial lipids

Bacteria, yeasts, moulds, and microalgae have all been employed as sources of lipids and enzymes in the past few decades. They were first used to manufacture laundry-grade lipases in the 1960s, which were used in conjunction with detergents and other cleaning agents. The introduction of microbial oil as a food supplement (i.e., as a source of gamma-linolenic acid) in the 1980s was followed by a flurry of other notable breakthroughs. Microorganisms are increasingly providing long-chain polyunsaturated fatty acids utilized as high-grade nutraceuticals for human (particularly newborn) and animal nutrition (Ezeji et al., 2007).

Over the last decade, microbial lipids have emerged as a potential resource for the long-term production of biofuels and value-added bioproducts as an alternative (and replacement) for harmful petro-based chemicals. Several bacteria (also known as oleaginous microorganisms) have the potential to accumulate enormous amounts of lipids when given the right conditions. Oleaginous are microorganisms that have > 25% lipids in their cell biomass (Ezeji et al., 2007). Several microorganisms contain sufficient oil content to be used for oil production (oil content, % dry weight), including (1) microalgae (*Botryococcus braunii*, 25%–75%; *Neochloris oleoabundans*, 35%–54%; *Chlorella* sp., 28%–32%; and *Schizochytrium* sp., 50%–77%); (2) bacteria (*Arthrobacter* sp., > 40%; *Acinetobacter calcoaceticus*, 27%–38%; and *Rhodococcus opacus*, 24%–25%); (3) yeasts (*Cylindrotheca* sp., 16%–37%; and *Rhodotorula glutinis*, 72%); and (4) moulds including *Aspergillus oryzae*, 57%; *Mortierella isabellina*, 86%; *Humicola lanuginosa*, 75%; and *Mortierella vinacea*, 66% (Ratledge, 2004).

Bacteria begin to produce lipids when the carbon supply is abundant and other nutrients (mainly nitrogen) are depleted in the culture media. The culture medium must have a high carbon-to-nitrogen ratio to enable favorable lipid accumulation. Excess carbon in the cell is converted to triacylglycerol, which is a type of lipid. With the presence of excess nitrogen and other nutrients, Wu et al. (2010) demonstrated lipid biosynthesis in the oleaginous yeast *R. glutinis* using wastewater as a raw material, suggesting that phosphate and sulphate limitation can also drive lipid accumulation in *R. glutinis*. With lipids ranging from 4% to 68%, a range of low-cost materials (such as cheese whey, wheat bran, and sewage sludge) are presently being researched in an attempt to minimize the cost of lipid synthesis (Zeng et al., 2013).

Limited lipid yields, minimal mass transfer, and the production of stable emulsions are all important challenges when extracting lipids from microbial biomass. Several approaches have been examined, and co-solvent approaches have shown to be effective for total lipid accessibility at the experimental level. However, more research is needed to determine how they may be used in industrial biofuel production. Solvents with the qualities capable of evaluating lipid recovery from microbial biomass are required in the future in commercial biofuel industry (Zeng et al., 2013).

7. The benefits and prospects of microbial biofuel

Biofuels are a renewable energy source. If carbon imprisoned in fossil fuels is reintroduced into the carbon cycle today, the generation of GHGs, which traps heat in the atmosphere, is expected to produce detrimental consequences on the climate. GHGs are created in a variety of ways, including the burning of plant and fossil fuels for power production and transportation (Intergovernmental Panel on Climate Change, 2007), which produces anthropogenic CO₂; animal husbandry, which creates methane; and deforestation, which reduces the available natural carbon sinks (Intergovernmental Panel on Climate Change, 2001). Since the transportation industry expands at a 3% annual pace

(U.S. Energy Information Administration, 2010) and emits the greatest amount of GHGs, this might be mitigated by employing renewable energy sources, such as biofuels.

Microorganisms may ferment a wide range of carbohydrates to produce several different fuel molecules, including those utilized in transportation and power generation. Microbial biofuels have the added benefit of being both renewable and environmentally beneficial (Gientka et al., 2017). By utilizing the ability of diverse microorganisms to exploit and break down agro-industrial wastes, such as bagasse, molasses, stover, grain husks, and seed cakes (*J. curcas* seed cake) (Kumar and Kumar, 2017; Kumar et al., 2018; Madakka et al., 2021), microbial biofuel production technologies reduce environmental waste while lowering GHG emissions.

Microorganisms are everywhere, and they may be controlled in a variety of ways to improve specific features. Several countries are considering biofuels as a replacement for fossil fuels to lessen their dependency on oil imports (Madakka et al., 2021).

Most of these fuels were alcohols derived from starch or sugar fermentation or essential oils (Antoni et al., 2007). Microorganisms can convert nearly any type of biomass into compounds that can be utilized as transportation biofuels. Despite the fact that ethanol, methane, butanol, and other biofuels can be produced almost as efficiently as crude oil refining products, this method is not only to blame for global warming, but also hazardous to the environment (Kumar and Kumar, 2017).

8. Ethanol production processes

A variety of sugars can be used as a raw material for bioethanol production in industrial fermentation. These materials are divided into three categories, sugar yield i.e., cane, wheat, beetroot, fruits, and palm juice; starchy yield (e.g., grain) obtained from root plants (e.g., potato and cassava) and crops, such as wheat, barley, rice, sweet sorghum, and corn; and cellulosic biomass, which includes wood and wood debris, cedar, pine, and agricultural leftovers and fibers (Kumar and Kumar, 2017; Konur, 2021).

Bioethanol and biomass ethanol are both alcohols produced via a biochemical process from lignocellulosic biomass (such as rice straw and wheat straw residue). Traditional processing is impossible to ferment starch or amyllum containing a large number of glucose polymers due to their chemical composition (Kumar and Kumar, 2017). It can be hydrolyzed to the simplest glucose chains by combining 15%–20% starch with water and heating the mixture to a high temperature, followed by treatment with hydrolyzing enzymes. Amylase is a well-known enzyme that breaks down hydrolyzed starch molecules into simple glucose chains during the liquefaction process. After cooling to approximately 30 °C, microorganisms are added to ensure fermentation (Konur, 2021).

The enzymatic hydrolysis of various carbohydrates, such as maize and wheat, produces ethanol. The following are the reasons why dry and wet mill procedures are used in the maize ethanol sector: (i) ethanol dry mills have a lower capacity and are primarily designed for the manufacturing of ethanol as well as ethanol and animal feeds and (ii) the new wet mill unit can generate 1 gallon of ethanol using 35150 British thermal unit (Btu) and 2134 kW per hour (KWh) of electricity as well as a variety of other useful products, such as organic acids, and solvents (Minteer, 2016; Konur, 2021).

9. The fermentation industry

The fermentation process is the metabolic pathway of organic compounds by bacteria, which involves chemical changes caused by enzyme activity. According to the presence of oxygen, fermentation processing can be divided into two categories: aerobic and

anaerobic (Khandaker et al., 2020). Several microorganisms found in nature have the ability to undergo fermentative modifications and, some of them, such as yeasts, bacteria, and moulds, are capable of producing ethanol using a variety of carbohydrate polymers. In general, microorganisms break down distinct carbohydrate molecules in aerobic and anaerobic environments during the fermentation process, culminating in the generation of ethanol (Khandaker et al., 2020).

A fermentation efficiency of 46% converts approximately 40%–48% of fermented glucose to ethanol, implying that 1000 kg of sugar in the fermentation industry can produce 583 L of pure ethanol (Siqueira et al., 2020).

10. Anaerobic digestion

The anaerobic digestion of biomass has received substantial attention as a potential alternative approach for producing fuel and biofertilizer through organic farming. By breaking down organic substrates and creating methane and CO₂ gases at a ratio of 60%–70% methane and 30% CO₂, the same procedure as for biogas production is used in this application (Sikora et al., 2017). Consequently, the anaerobic digestion of biological waste is seen as a promising processing method for biofuel generation (Sikora et al., 2017).

Anaerobic digestion can be utilized to produce methane from municipal solid waste as a first step. These molecules can be combined with nitrogen from the atmosphere, CO₂ from the environment, and remnants of organic substrate, also known as landfill gas. Each pound of biodegradable organic waste can produce 10–12 standard cubic feet of biogas (Siqueira et al., 2020). Methane, on the other hand, has the same low quality as natural gas due to the need to remove volatile organic contaminants and CO₂ in order to make a high-quality commercial product (Siqueira et al., 2020).

Extremely efficient separation equipment is required to manufacture natural gas from landfill gases. Internal combustion engines, turbines, microturbines, direct use in boilers, dryers, furnaces, and home ovens all have significant potential for generating energy from gas-producing facilities. Due to the high expense of manufacturing and purification, there has recently been a renewed focus on investigating the use of gas contained in the ground as a fuel-generating liquid rather than gaseous fuel produced by anaerobic fermentation (Siqueira et al., 2020). Some of the benefits of liquid methanol production includes a low sulphur concentration, low ash content, and can be commercially utilized. Liquid fuel is easier to carry and store than gaseous fuel. Lignocellulosic biomass is used in liquid fuels and agricultural biofertilizers owing to the anaerobic fermentation process (Siqueira et al., 2020).

11. Whole crop biorefinery

Seeds are utilized as raw materials and intermediates in biorefineries to obtain useful products from oil crop biorefinery processes. As a result, the oilseed plant, *J. curcas* has been used as a nucleus, holding the seeds of *J. curcas* oil at 35%–40%, with a potential yield of 1–1.5 tons of oil per hectare (Setyobudi et al., 2017).

To convert biomass into energy, the valuable parts of the biomass can be divided into several components, which can then be independently treated. Following biomass separation, the oil yield is used as the primary (or basic) feedstock for biodiesel synthesis, or it can be chemically processed to produce a variety of oleochemical products. Solid materials, on the other hand, is employed in the creation of primary crude materials for chemical structure composition or biological gas composition. Lignocellulosic feedstock obtained from refineries can be used as a starting substrate for biorefineries to boost gas output (Naik et al., 2010).

12. Frequently used microorganisms in biofuel production

S. cerevisiae, generally known as baker's yeast, is the most extensively utilized microorganism in the fermentative processing of biofuels. A wide variety of species are employed in the production of biofuels. Several of these species have been bioengineered to produce more biofuel than they are capable of producing naturally (Khandaker et al., 2020).

13. Limitations to the production and use of microbial biofuels

The most significant barrier to the application of microbial biofuels is the ethical considerations. Biofuel programmers are concerned that they have increased global food costs and will continue to do so as firms and governments divert agricultural supplies to supply electricity and transportation fuels. On the other side, a greater concentration on secondary biofuel production is helping to minimize this (Siqueira et al., 2020). While there are several other barriers to the development of secondary biofuels, the change from food to biomass-based feedstock for bioenergy production might significantly reduce the food versus fuel dilemma. Biomass feedstock are being used to produce livestock feed (Srivastava et al., 2018).

Grass and certain crop wastes can be used as cattle fodder (such as corn stover). Cattle pasture is typically provided in less productive, prone to erosion, or arid conditions; the same landscapes are being examined for biofuel feedstock production (Rodionova et al., 2017). As a result, biofuel producers may face competition for herbaceous feedstock from animal farms (grasses and crop residues). Expensive pretreatment techniques have hampered the growth of second-generation biofuels by raising the costs of production. Using *Trichoderma reesei* cellulases to hydrolyze cellulose costs were approximately 2.5–5 cents for every liter of ethanol produced (Boboescu et al., 2016; Rodionova et al., 2017; Srivastava et al., 2018).

Biodiesel production has a low land yield and competes with agricultural land that may be used to grow food. Consequently, the high cost of biodiesel is a major barrier to its adoption. The oil is inedible due to its anti-nutrient components, and its use in non-biodiesel manufacturing does not compete with food. This is the most essential benefit of *J. curcas* oil (Ma et al., 2018). University of Ilorin, the largest research plantation in Nigeria, has set aside hectares of land for the research and production of *J. curcas* oil-based biodiesel. Adapting the continuous transesterification process and recovering high-quality glycerol from glycerol wastes are two significant ways being investigated now to reduce biodiesel costs and pave the way for sustainable local biodiesel production (Christophe et al., 2012; Ambat et al., 2018; Ma et al., 2018). In addition to the high cost and energy requirements of the transesterification process and the subsequent separation of biodiesel from glycerol, there is a scarcity of low-cost vegetable oil feedstock (Ambat et al., 2018).

Several studies have focused on enzymatic transesterification using lipase and whole-cell biocatalysts technology for biodiesel synthesis from microalgae to overcome these limitations (Connor and Liao, 2009). Additionally, not every automobile owner is eager to utilize ethanol in their vehicle; as a result, ethanol in gasoline/ethanol mixes has been met with skepticism and criticism in some parts of the world (Sakuragi et al., 2011). Drivers in Germany, for example, defied a government-imposed speed limit by blocking a government-mandated ethanol mix attempt with a large-scale active boycott, putting the German government's ambitious biofuel program on hold. As a transportation fuel, bioethanol is not as efficient as gasoline. Excessive alcohol in alcohol fuel blends

is reported to damage the aluminum fuel system components (Sakuragi et al., 2011).

The use of biobutanol, which is less corrosive than ethanol and does not involve the modification of existing equipment, such as pumps, tanks, and pipes, mitigates these shortcomings (Sakuragi et al., 2011). Biobutanol is a better fuel molecule than ethanol since it can completely replace gasoline or be blended with it at any quantity, whereas ethanol can only be blended up to 85% (Amaro et al., 2011).

The EU, which is also the largest biodiesel consumer, produces 82% of all biofuels. Attempts to produce oil-bearing crops for the production of biodiesel for the EU market have been made in Africa. Some have proved disastrous, such as the ambitious *J. curcas* biodiesel project by Sun Biofuels in the Kisarawe District, Tanzania, which was allocated an estimated 8000 ha of land. The people were left penniless, jobless, and without ancestral land to live because the project never transpired. Growing demand for biofuels in the United Kingdom and the EU has encouraged British enterprises to take the lead in Africa. Eleven British firms were linked to half of the 3.2 million hectares of biofuel land identified (Shanmugam et al., 2020).

Up to 6 million hectares have been gained across Africa, according to ActionAid. However, there is considerable potential for abuse because most landowners are uneducated and unaware of their rights (Shanmugam et al., 2020). Similar challenges exist in other countries, such as Nigeria, wherein naive farmers are tricked into producing and providing the EU with "special" varieties of *J. curcas* (Amaro et al., 2011; Shanmugam et al., 2020).

14. Future trends in microbial biofuel production

The primary focus of biofuel science will be the genetic engineering of microorganisms to attain increased product specificity and production. Higher-level biofuels will become more important as the fight over food versus fuel heats up. The issue of lignocellulose degradation will take the front stage. The following developments are expected to have a positive impact on microbial biofuel production, affordability, and productivity in the near future (Ventorino et al., 2015).

15. The sustainable use of renewable energy

Search for alternative aviation fuels is a collaboration led by the Cranfield University in the United Kingdom, which comprises Airbus and British Airways, among others, intending to discover alternative aviation fuels. The project was designed to develop microalgae quicker than any other attempt while also trapping CO₂ from the atmosphere and ocean (Akhundi et al., 2019).

The most important advantage of this project is that it will not interfere with agricultural land, will not require freshwater, will not result in deforestation, and will not impact the ecosystem (Akhundi et al., 2019). KLM Airlines' SkyNRG is another of such consortium (Akhundi et al., 2019). DISCO, an EU-funded initiative, is looking for microorganisms that can break down lignocellulosic materials. It consists of research institutes, universities, and corporate partners (USDA, 2009).

The ultimate goal of the biofuel research is to develop a cocktail of microorganism-derived enzymes that can break down complicated lignocellulose into simple sugars while also permitting yeast co-fermentation to produce bioethanol (Ventorino et al., 2015). Through the government-owned industrial development corporation and the central energy fund, the South African government intends to boost microbial biofuel production (USDA, 2009).

Rainbow Nation Renewable Fuels Limited has announced the construction of an R1.5 billion (\$0.18 billion) biofuel processing

plant in Eastern Cape, South Africa. The business will process 1.36 million tons of soybeans per year and produce 288 million liters of biodiesel, making it the largest soybean processor in Africa (USDA, 2009). Several scientists now agree that commercial biofuel production may be reconciled with feeding humankind while also conserving the environment, if we put in the time and effort required to make the necessary improvements (Strauss, 2019).

Engineered host fermentations incorporating heterologous genes in *Escherichia coli* and *S. cerevisiae* have also been demonstrated to be involved in biodiesel fatty acid ethyl ester production. In addition, researchers have isolated *R. opacus* from soil polluted with petroleum waste products, a bacterium with a flexible appetite capable of consuming a variety of sugars and toxic compounds and converting them to triacylglycerols, which can then be used to produce biodiesel (Yuan et al., 2020).

McCarthy (2012) reported that scientist's isolated genes needed to metabolize alginate by the marine bacterium, *Vibrio splendidus*, and spliced them into *E. coli*, resulting in a new strain of *E. coli* capable of metabolizing alginate, the major sugar in seaweed (macroalgae). This novel strain turns 28% of the seaweed's dry weight into ethanol, resulting in an ethanol output of 80% of the theoretical potential output. This is a significant improvement because natural seaweed species grow at 10 times the rate of conventional plants and are abundant in sugars. This most recent development holds a great deal of potential for the future (Doi et al., 2017).

Hydrogen gas is considered a potential energy source because it is renewable, produces only water as a byproduct, and does not emit CO₂. During burning, it releases a substantial amount of energy per unit weight and is easily converted to electricity by fuel cells. Hydrogen can be produced by fermentative bacteria, photosynthetic bacteria, and algae (Lee et al., 2019).

Isobutanol is also being looked into as a possible new biofuel. It is a branched C4 and C5 alcohol that could be utilized as a gasoline replacement. Isobutanol is identical to n-butanol; however, isobutanol has a higher octane number and is slightly less expensive than gasoline (Hosseini and Wahid, 2016; Lee et al., 2019).

Conversion systems must be improved when scaling systems for scattered processing to increase yield and reduce costs. Researchers are investigating pretreatment technologies as well as microorganisms and enzymes that deconstruct carbohydrate polymers and produce long-chain hydrocarbons or alcohols to strengthen the biochemical platform (Stephen and Periyasamy, 2018). The goal of thermochemical research is to develop integrated thermo-catalytic processes that can shift between different feedstock. Future conversion systems must maximize the value of items generated, reduce energy and water use, scale to distributed processing networks (to address feedstock logistics concerns), and generate as little waste as possible (Stephen and Periyasamy, 2018).

Companies, such as Solazyme, are developing microorganisms (native or genetically modified) that can ferment glucose into a variety of infrastructure-friendly, energy-dense, fourth-generation biofuels, such as longer chain alcohols, alkanes, and alkenes. Xylose fractions are more challenging to work with because fewer bacteria have the necessary metabolic machinery (Saha and Cotta, 2007).

H₂- and algae-derived oil will almost certainly spark further research. Microalgae, which have the highest yield per acre of all the sources, have the potential to become the most important energy source in the future. Microalgae are being advertised as a potential third-generation biofuel feedstock due to their rapid growth rate, ability to fix CO₂, and high lipid production capability. Moreover, they can be farmed on nonarable lands and do not compete with food or feed crops. Microalgae have a considerable bioenergy potential since they can produce biodiesel and

bioethanol, which are liquid transportation and heating fuels (Parvez et al., 2020).

The Biotechnology and Biological Sciences Research Council financed a study at the University of Cambridge that discovered and analyzed the genes for two enzymes that toughen wood, straw, and stalks, making it harder to extract sugars for ethanol biofuel manufacture. The researchers examined the *Arabidopsis* plants that were missing two of the enzymes required to make xylan from lignocellulose (Eckert and Trinh, 2016). When extracting biofuel from these plants, the researchers discovered that converting 100% of the xylan into sugar needed less effort. New bioenergy crop varieties with these characteristics, such as willow and miscanthus grass, are being developed (U.S. Department of Energy, 2016).

A company called Algenol is working on new technology for producing bioethanol from algae. Rather than producing algae and then harvesting and fermenting it, sunlight is used to immediately produce ethanol, which is then removed without harming the algae. The approach can yield 56,000 L of bioethanol per hectare per year, according to the business, compared to 400 (3750 l/ha) for maize production (U.S. Department of Energy, 2016).

16. Conclusion

The production of biofuels from microorganisms has greatly advanced since Louis Pasteur discovered butanol synthesis by bacteria in 1862. The variable nature of petroleum pricing as a result of political unrest and foreign policy as well as the detrimental impacts of GHGs generated by the burning of petroleum products has reignited interest in finding a long-term solution. Biofuels produced by microorganisms are continually being developed or modified to minimize dependency on finite fossil fuel supplies, create jobs, and contribute to a healthier planet. Various species have been genetically modified. *Clostridium acetobutylicum*, *R. opacus*, *S. cerevisiae*, and *E. coli* were examined to increase product specificity and production. More research is being performed to help limit the creation of undesired by-products, increase the price and performance of biofuels in comparison to fossil fuels, and lessen the impact of biofuel production on food costs. The possibilities for microbial biofuel generation are infinite, and hydrogen and other fuels can probably be extended as aviation fuels. This has the potential to not only attenuate, but even reverse the harmful consequences of climate change in the long run.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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