**FUTURE TRENDS IN RENEWABLE AND SUSTAINABLE ENERGY RESOURCES: USING ESSENTIAL MICROBES FROM INDUSTRIAL SECTOR**

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**Abstract:**

The search for renewable and sustainable energy sources has become imperative as the world continues to grapple with the challenges posed by climate change and the depletion of finite fossil fuel reserves. Biofuels are derived from organic matter and have gained traction as an eco-friendly substitute for conventional fossil fuels. By utilizing feedstock’s such as agricultural residues, dedicated energy crops, and even algae, biofuels offer reduced greenhouse gas emissions and can be compatible with existing combustion engines. Researchers are actively investigating methods to enhance biofuel production efficiency and address land-use competition, food security, and biodiversity concerns. Biodiesel, a type of biofuel, is produced through the trans-esterification of vegetable oils or animal fats. It is a renewable and biodegradable alternative to traditional diesel fuel, with the potential to decrease greenhouse gas emissions significantly. Hydrogen is regarded as a versatile, clean energy carrier with zero carbon emissions when utilized in fuel cells. It can be produced through various processes, including water electrolysis using renewable energy sources like wind, solar, or hydropower. Developing hydrogen infrastructure and storage solutions is vital to unlock its potential as a mainstream fuel for vehicles, industrial applications, and power generation, aiming to create a sustainable and emissions-free energy landscape. Cyanobacteria, represent a promising avenue for sustainable energy and chemical production. These microorganisms can efficiently convert sunlight and carbon dioxide into biofuels and valuable biochemical through photosynthesis. While each renewable resource offers distinct advantages and challenges, they share the common goal of fostering a sustainable energy transition. Continued research, technological advancements, and supportive policies are essential to realizing the full potential of biofuel, biodiesel, hydrogen, and cyanobacteria as key components of a cleaner and more environmentally conscious energy future. Embracing these renewable alternatives presents a promising pathway to mitigate climate change and promote global energy security. This review focuses on promising alternatives that have garnered significant attention recently: microbial fuel cells, biofuel, biodiesel, hydrogen, and cyanobacteria.

**Keywords:** Biofuels; Sustainable; Cynobacteria; Renewable; Microbial.

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**1. Introduction**

The consumption of energy increased in recent years. In developed countries, 28% world’s population uses 77% of energy production (Desa, 2019). By 2050 it was estimated that the population will reach 9.7 billion, and developing countries witness 90% of the population growth. Microbes produce energy from organic and inorganic substances through various metabolic pathways to produce different types of biofuels. Apart from photosynthesis, certain microbes like cyanobacteria decompose water into desirable oxygen and hydrogen (Agyekum et al., 2022). Some of the microbes have the ability to break down environmental pollutants into valuable energy compounds (methane and alcohol) (Teng et al., 2019). By understanding the metabolic pathway and phenotypic makeup of a particular microbe the desirable end product can be obtained through efficient technologies (Fabris et al., 2020). The selection of desirable substrates plays a vital role in microbial biofuel production. Present energy production involves financial support, remediation strategies, and initial research. Natural pollutants affect the benefits of fossil fuel energy where CO2, NO2, and SO2 become harder to remediate (ÓhAiseadha et al., 2020).

To overcome environmental pollution like soil, air, and water cost-effective biorefineries must be produced (Zetterholm et al., 2020). The optimal production of biofuel depends on the selection of particular microbes and their suitable substrate. Microalgae and Cyanobacteria reduce atmospheric CO2 into biofuels photosynthetically whereas methanotrophs utilize methane to produce methanol (Liao et al., 2016). Bacteria like *Geobacter sulfurreducens* and *Shewanella oneidensis* transfer an electron to other conductive surfaces via the outer membrane which is useful in bioelectricity generation (Kracke, 2015). In the absence of oxygen metal-reducing microbes use metal salts as electron acceptors. The present chapter demonstrates how microbes can be utilized for clean energy production which can be renewable and sustainable in the future.

**2. Microbes and Biofuel**

Biofuel having more positive net energy is considered for commercialization. Biomass with higher lignocellulose content (plant biomass) is an efficient substrate for microbes. *Saccharomyces cerevisiae* cannot degrade lignocellulose completely (Chang et al., 2013). The processing of lignocellulolytic plant biomass starts with pre-treatment followed by enzymatic bioprocessing (Kumar et al., 2009). The cellulolytic breakdown of plant biomass can be by physical, biological, or chemical means. The pretreated biomass undergoes hydrolysis by using microorganisms or a mixture of enzymatic treatments (Lynd et al., 2002). Gases like methane are emitted in lesser quantities compared to other greenhouse gases and are more potent than CO2 (Yvon-Durocher et al., 2014). Methane forms the major component in natural gas which can be transformed into biofuel (methanotrophs) or into methanol (Liao et al., 2016). A current advantages and disadvantages of various biofuels production was schematically represented in **Figure 1**. Methane gas can be produced either biologically or non-biologically. Most of the methane is produced in a biological way with the help of microorganisms, especially methanogenic archaea via the methanogenesis pathway (Conrad, 2009; Prathiviraj et al., 2019; Chellapandi and Prathiviraj, 2020; Prathiviraj and Chellapandi, 2020a). The end product of industrial microbes for biofuel productions was detailed infer in the **Table 1**.

**Table 1** Types of biofuel substance produced by beneficial microbes (Kumar and Kumar, 2017).

|  |  |
| --- | --- |
| **Biofuel** | **Microorganism** |
| Ethanol | *Escherichia coli* |
| *Zymomonas mobilis* |
| *Caldicellulosiruptor bescii* |
| *Trichoderma reesei* |
| *Saccharomyces cerevisiae* |
| *Candida shehatae* |
| *Fusarium oxysporum* |
| Butanol | *Clostridium acetobutylicum* |
| *Escherichia coli* |
| *Pseudomonas putida* |
| Isobutanol | *Clostridium thermocellum* |
| 2, 3-butanediol | *Zymomonas mobilis* |
| Fatty acid | *Yarrowia lipolytica* |
| *Saccharomyces cerevisiae* |
| Isoprenoid based-biofuel | *Saccharomyces cerevisiae* |
| Limonene | *Synechococcus sp.* |
| 1, 3-propanediol | *Synechococcus elongates* |
| Lipids | *Cryptococcus vishniaccii* |
| *Acinetobacter calcoaceticus* |
| *Arthrobacter sp.* |
| *Bacillus alcalophilus* |
| *Rhodococcus opacus* |
| *Candida curvata* |
| *Cryptococcus albidus* |
| *Lipomyces starkeyi* |
| *Rhodotorula glutinis* |
| *Trichosporon oleaginosus* |
| *Aspergillus oryzae* |
| *Humicola lanuginosa*  |
| *Mortierella isabellina* |
| *Mortierella vinacea* |

Methanogens lack peptidoglycan in the cell wall structure. Alternatively, they possess heteropolysaccharide (*Methanosarcina)*, pseudomurein (*Methanobacterium* and *Methanobrevibacter*) (Hook et al., 2010). *Methanobacteriales ruminantium* found in ruminants produce methane in the presence of coenzyme M (terminal methyl carrier), Coenzymes F420 (dehydrogenase enzyme) hydrogen, and carbon dioxide (Chellapandi et al., 2018; Prathiviraj and Chellapandi, 2019; Prathiviraj and Chellapandi, 2020b; Srivastava et al., 2020). One billion tons of methane was produced and consumed by microorganisms each year. The removal of methane occurs in Earth’s atmosphere (troposphere and stratosphere) with the help of ultraviolet radiation in a non-biological way (Wang et al., 2021). In the catalytic steam reforming process methane is turned into synthetic gas containing hydrogen and carbon monoxide in the presence of nickel as the catalyst at 700 – 1100 ℃. Which can be used as a raw material for the production of hydrogen and methanol (Meloni et al., 2020). The enzyme monooxygenase reduces the oxygen to peroxide and then to methanol in the presence of methane (de Souza et al., 2022). The conversion of coal into methane is carried out synergistically by a syntrophic community of microorganisms *Clostridium*, *Enterobacter*, *Klebsiella*, and *Citrobacter* (Rodríguez-Reyes et al., 2021).

The most common biofuel available today is bioethanol which is produced through the fermentative pathway. Ethanol can be produced by various types of microorganisms. Globally 29 billion gallons were produced by the year 2019. The United States and Brazil were the top producers of bioethanol (84%) (Tse et al., 2021). In the presence of yeast, the biomass undergoes an oxidation reaction followed by decarboxylation of pyruvate to form ethanol (Lin et al., 2018). *Saccharomyces cerevisiae* is much more efficient compared to *Escherichia coli* in producing ethanol through direct decarboxylation. The most efficient way of producing ethanol is in the absence of Co-enzyme A (CoA), which is possible in genetically engineered microorganisms. Artificial metabolic pathway designing requires specific tools to help the mRNA and proteins to be functional in the designed pathway (Koppolu and Vasigala, 2016). *Zymomonas mobilis* produces a higher ethanol yield than *Saccharomyces cerevisiae*. Genes encoding for enzymes such as mannose and xylose tend to increase the bio-ethanol yield to 89.9 % in 72 hrs (Yang et al., 2016). Bio-ethanol production from lignocellulosic raw material is high so microorganisms that can synthesize ethanol from glucose and xylose are used (Ruchala et al., 2020).



**Figure 1** Advantages and disadvantages of various biofuels (Talapko et al., 2022).

The biomass of microalgae is converted into a gaseous state known as syngas, which is a mixture of methane, CO, CO2, nitrogen, and hydrogen (Chellapandi et al., 2017; Poudel et al., 2019). Syngas is used for producing methanol, ethanol and synthetic hydrocarbons, butanol, methane, butyric, and acetic acid. It is also used as a turbine fuel (Ciliberti et al., 2020; Sangavai et al., 2020). Metals like Zinc, Iron, Magnesium, and nickel can be found in microalgal bio-oils which are removed by heat treatment (Znad et al., 2022). A schematic representation of production of biofuel from various sources was depicted in **Figure 2**.



**Figure 2** Production of biofuel from various sources (Kumar et al., 2017).

**2.1 Biodiesel**

The first alternative and well-known biofuel is biodiesel. Which is obtained by the transesterification of oil and fat (Knothe and Razon, 2017). Due to the increased consumption of energy (directly or indirectly) and to reduce the emission of CO2 usage of biodiesel is preferred (Leach et al., 2020). About 53% (105 billion liters) of the total consumption of biodiesel in the world comes from European Union (Popp et al., 2016). Plants like grape and soybeans contain different types of nitrogen fixatives like *Azotobacter sp*. and *Azospirillum sp*., *Acinetobacter junii* and *Pseudomonas fluorescens* act as phosphofixatives. They benefit the plant through the rhizome system (Hindersah et al., 2020). Through nitrogen fixation, the plants produce lipids and triglycerides as end products (Liu et al., 2018). Microbial oil can be produced from *Rhodotorula glutinis* and *Yarrowia lipolytica* by transesterification. Certain types of bacteria and fungi have a greater tolerance for higher triacyl glycerol (Caporusso et al., 2021). Bacteria are more efficient than fungi in microbial oil production due to their adaptations and easy maintenance. *E.coli* synthesis biodiesel in the form of fatty acid esters also ferments biomass that is from carbon sources that are renewable (Zabermawi et al., 2022).

Biodiesel serves as the replacement for petroleum-based fuels. Fatty acids undergo transesterification of fatty acids with short-chain alcohols and form long-chain fatty acids with long-chain esters (Jafarihaghighi et al., 2020). Genetic engineering techniques can assist in the production of biodiesel with higher efficiency. Microalgae are the major raw material for the production of biodiesel due to their higher fatty acid content (Gouveia and Oliveira, 2009). The process of pyrolysis is applied to remove extra residues (pollutants) of microalgae after lipid extraction at a temperature of (~700℃) (Huang et al., 2022). Some of the common microalgaes were *Botryococcus braunii, Chlorella vulgaris, Crypthecodinum cohnii, Dunaliella primolecta, Navicula pelliculosa, Scenedsmus acutus, Crypthecodinium cohnii, Monallanthus primolecta, Monallanthusocornussel olia*, and *Teallanthus chlorideasul*. Their lipid content varies from low 1% to high 70%. The growth medium consists of fixed pH, speed, mixing, dissolved oxygen and CO2, intensity, and wavelength of the light source (Dolganyuk et al., 2020). Depending upon the bioprocess in the bioreactor the lipid production by the microalgae is determined (Subramaniam et al., 2020). The microalgal residues contain carbohydrates that can be used for ethanol production through fermentation (Ebhodaghe et al., 2022). A detailed schematic representation was represent for process of biodiesel production and recovery of methanol in **Figure 3**.

**2.2 Hydrogen**

Biohydrogen can be used as a substitute for fossil fuels. During combustion the produces water rather than carbon monoxide and carbon-di-oxide (Osman et al., 2022). Biohydrogen has an energy value is 142 MJ/kg, which is higher than natural gas 52 MJ/kg, and petrol 44 MJ/kg (Osman et al., 2022). By 2024 the demand for hydrogen fuel will increase to 120 million tons and it also fulfills the goal of clean and affordable energy (Sharma et al., 2021). In anaerobic condition, microorganism uses oxidation-reduction potential which produces sulfides, nitrides, and phosphides from hydride which produces enough energy to initiate a reaction process (Chen et al., 2020). By creating joint metabolism between syntrophic communities better microbial refineries can be produced. Methanogenic bacteria transfer hydrogen and formate between syntrophic partners (Greening et al., 2019). Carbon-based biogas protects the environment from unwanted gases like sulfur.



**Figure 3** Process of biodiesel production and recovery of methanol (Talapko et al., 2022).

The process of production of biohydrogen can be from organic matter or wastewater (Moussa et al., 2022). Electron transfer to conductive surfaces can be initiated by creating an electro-biofilm where the gathered electrons can be used for producing hydrogen and electricity. Under anaerobic conditions, microalgae convert water into hydrogen and oxygen (photolysis) (Wang et al., 2021). Hydrogen ions can be produced in two ways by photosynthesis, one is through separate acid and hydrogen production. In the second method, microalgae are forced to survive in the presence of starch rather than sulfur which produce hydrogen ions (Burlacot and Peltier, 2018). The net production of hydrogen is less through this process yet there are zero harmful byproducts produced. Raw materials like cotton-sludge hydrolates and lignocellulosic materials were commonly used for biohydrogen production. The starter cultures for these raw materials were isolated from fish and termites (Bhardwaj et al., 2021). Microorganisms like *Enterobacter, Klebsiella, Clostridium,* and *Citrobacter* were commonly used (Bhatia et al., 2021).

Microorganisms like *Geobacter sulfurreducens* and *Shewanella oneidensis* have a unique molecular mechanism that pulls electrons from the outer membrane to the surface. Bioelectrochemical cells (BECs) produce bioenergy from bioenergy from biomass and wastewater (Li et al., 2015). The energy released from microbial electrolysis cells (MECs) and microbial fuel cells (MFCs) is not sufficient for commercialization (Santoro et al., 2020). Bio-hydrogen can be produced fermentatively by strict anaerobes like *Clostridium sp*., methanogenic facultative anaerobes. Hydrogen produced by the fermentative pathway is more efficient and profitable when compared with the photosynthetic pathway (Santoro et al., 2020). This is due to the rapid growth of fermentative bacteria under anaerobic conditions and the presence of oxygen doesn’t affect higher levels of hydrogen production (Vardar‐Schara et al., 2008). One mole of glucose yields 12 moles of hydrogen but practically 3.8 moles of hydrogen can be yielded. By using mixed starter cultures and combined metabolic pathways the yield can be increased (Wang et al., 2021).

**3. Cyanobacteria**

Cyanobacteria are photosynthetic prokaryotic organisms that produce biofuels and biodegradable plastics. Due to the presence of fatty acid content and growth rate, they can be used as a better alternative to other resources (Gouveia and Oliveira, 2009). Cyanobacteria produces diacylglycerol (DAG) and triacylglycerol (TAG) which acts as the precursor for biofuel production. The cyanobacterial genetic sequence was well known which makes them more efficient for the production of biofuels (Savakis and Hellingwerf, 2015). 10% of the solar energy can be stored in the form of biomass whereas eukaryotic algae store 5% and 1% by energy crops (Parmar et al., 2011). Photosynthetically cyanobacteria outperform plants and algae. *Synechococcus elongatus* PCC 7942 and *Synechocystis sp*. PCC 6803 is commonly used as a biofuel source. The lipids present in the cyanobacteria are present in the thylakoid membranes. The cyanobacterial strain *Synechococcus elongatus* PCC 7942 contains genes encoding for alcohol dehydrogenase and pyruvate decarboxylase which helps the cyanobacteria to transfer carbon from pyruvate to ethanol (Deng and Coleman, 1999).

Hydrogen ion that is produced during photosynthesis is not taken up by the bacteria rather it is retained (Marshall et al., 2012). Cyanobacteria utilize the energy from hydrogenase present in the sugar molecule that is used in the photolysis of water molecules (Veaudor et al., 2020). The electron produced by various pathways can be diverted to synthesize more biohydrogen molecules by hydrogenase to photosystem. Extremophiles use hydrogen and oxygen from the atmosphere to produce water for their use (Gregory, 2009). By evolutionary adaptation, 400 species of microorganisms use energy sources like hydrogen and carbon monoxide for their metabolism. Bacteria like *Rhodobacteraceae, Flavobacteriaceae*, and *Sfhingomonodaceae* utilizes CO and hydrogen present in the seawater for their energy sources (Jordaan et al., 2020). *Sfinopyxis alascensis* utilizes hydrogen ions with the help of NiFe hydrogenase (Kruse et al., 2017).

**4. Microbial fuel cells**

Microbial fuel cells use chemical energy present in the organic substrates and convert it to electrical energy (Idris et al., 2016). Wastewater can be used in this process for creating electrical energy and also helps in wastewater management. Some of the microorganisms are known as “Exoelectrogens” where the bacteria act as a biocatalyst that produces electrons (Juan, 2014). The electrons thus formed move towards the cathode along with hydrogen ion which comes into contact with oxygen to form water molecule resulting in the production of green electricity (Zhang et al., 2016).

When acetate is used as the basic substrate in MFC, acetate undergoes an oxidation reaction in the anode region to produce electrons and protons. An electrochemically active carrier transfers electrons into the cathode through the cell membrane (Li, 2013). An external integrated circuit takes the electron from anode to cathode (Kim et al., 2008). The neutrality of the charge is sustained due to the migration of the proton across the proton permeable membrane. Whereas the membrane is not permeable to electrons. The Proton exchange membrane separates the anode and the cathode compartments. This membrane also prevents the diffusion of oxygen from the anode to cathode (Flimban et al., 2018). The substrate acetate is broken down into CO2 and water molecules. The potential of the anode is -0.300 V and cathode is 0.805 V so the maximum cell potential theoretically is 1.105 V (Barua et al., 2018).

Based on the transportation of electrons to the electrode there are two types of MFCs. In mediator MFCs the corresponding bacteria activity mediates the transfer of electrons to anode (Dharmalingam et al., 2018). Some of the chemicals that help in the flow of electrons to anode are neutral red, humic acid, and anthraquinone-2, 6-disulphonate. Such chemicals are known as “electroactive metabolities” (Flimban et al., 2018). The presence of oxygen hinders the process of electron formation so it is highly recommended to go with anaerobic reaction in mediator MFCs. In mediator-less MFCs, there is no need for an external mediator. The help of nanowires that is seen in most of the bacteria present in the wastewater transports electrons to the electrodes (Logan et al., 2006). The mediator-less MFCs are comparatively non-toxic and less expensive. When mediator-less MFCs are operated there are certain things to be considered such as the application of redox enzyme for better electron transfer, a circuit with external resistance, an anode that bears fuel oxidation, the microbial activity must be reduced, and membrane-assisted transfer of proton towards the cathode (Flimban et al., 2018). Most of the mediator-less MFCs are designed based on microbial fuel cell-based and soil-based microbial fuel cells.

**5. Conclusion**

Sustainable energy resources are essential for addressing the environmental challenges associated with traditional fossil fuels. Four promising sustainable energy technologies are biodiesel, microbial fuel cells, cyanobacteria, and hydrogen. Biodiesel is a renewable and environmentally friendly alternative to conventional diesel fuel. It is produced from vegetable oils, animal fats, or recycled cooking oil, making it a sustainable choice. Biodiesel reduces greenhouse gas emissions and dependence on fossil fuels, making it an attractive option for transportation and some stationary power applications. However, its widespread adoption still faces challenges related to feedstock availability, land use, and competition with food production. MFCs are a fascinating technology that utilizes microorganisms to convert organic matter into electricity. They offer a sustainable approach to wastewater treatment and energy production simultaneously. MFCs hold significant potential in low-power applications, such as remote sensing devices or small-scale energy generation. Nevertheless, their scalability and efficiency remain areas of ongoing research, and further development is required to make them commercially viable for larger energy demands. Cyanobacteria are photosynthetic microorganisms capable of harnessing solar energy to produce biomass and, potentially biofuels. They offer a sustainable and renewable resource for energy production. Researchers are exploring ways to enhance their efficiency in converting sunlight into usable energy by genetically modifying cyanobacteria. However, challenges such as cost-effectiveness, scalability, and ethical considerations regarding genetic engineering must be addressed before widespread implementation. Hydrogen is considered a versatile and clean energy carrier, producing only water when used in fuel cells or combustion processes. It can be produced through various methods, such as electrolysis using renewable electricity or from bio-derived sources, ensuring sustainability. However, hydrogen faces challenges related to storage, transportation, and cost-effectiveness. Moreover, the energy required for large-scale hydrogen production should come from renewable sources to achieve its full sustainability potential. In conclusion, sustainable energy resources like biodiesel, microbial fuel cells, cyanobacteria, and hydrogen hold promise for a cleaner and more environmentally friendly future. However, their widespread adoption requires further research, technological advancements, and policy support to overcome current limitations and fully realize their potential to meet global energy needs while reducing environmental impact.

**List of abbreviations**

BECs: Bioelectrochemical cells

CO: Carbon monoxide

CO2: Carbon dioxide

CoA: Coenzyme A

DAG: Diacylglycerol

MECs: Microbial electrolysis cells

MFCs: Microbial fuel cells

NO2: Nitrogen dioxide

SO2: Sulfur dioxide

TAG: Triacylglycerol

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