THE IMPLEMENTATION OF WASTE BIOMASS SUBSTRATES AS FEEDSTOCK FOR THE PRODUCTION OF BIO-ELECTRICITY THROUGH MICROBIAL FUEL CELLS (MFCS): A SHORT REVIEW

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ABSTRACT

Lignocellulosic biomass plays a pivotal role in sustainable energy production, with a focus on indirect biomass fuel cells (IDBFC) and direct biomass fuel cells (DBFC). IDBFCs require the initial conversion of biomass into simpler forms like sugars, biogas, syngas, or biochar for subsequent electricity generation. In contrast, DBFCs offer a more direct approach, generating electricity from biomass without intermediate steps. Lignocellulosic biomass, composed of cellulose, lignin, and hemicellulose, has diverse applications, from bioethanol to direct electricity generation. However, the complex composition of lignocellulosic compounds, including carbon, hydrogen, oxygen, phosphorus, nitrogen, and sulfur, poses challenges for efficient enzymatic hydrolysis, a crucial factor in achieving high power density in Microbial Fuel Cells (MFCs). MFCs use microorganisms to convert substrates into electricity, influenced by factors like substrate degradation rate, circuit resistance, electron transfer rates, proton mass transfer, electrode materials, and operational conditions. The selection of proper electrode materials is vital for optimising MFC performance. At the heart of MFC performance are electricigens, microorganisms facilitating electron transfer from biomass to the anode through direct or indirect mechanisms. Direct electron transfer (DET), relying on physical contact between microorganism membranes and the anode, is preferred for its efficiency and eco-friendliness. The paper also explores the importance of nutrient supplements (macro and micro) in enhancing bio-methane production and process stability in agro-industrial biogas mono-digestion plants. Nutrient balance significantly affects microbial generation time, degradation rates, and gas production in anaerobic digestion processes. In conclusion, understanding the intricate interplay between lignocellulosic biomass energy fuel cells, electricigens, and their performance factors is crucial for advancing sustainable energy production. MFCs show promise in utilising sludge and various waste biomasses, positioning them as practical, reliable, and versatile power sources in the evolving landscape of renewable energy technologies.

Keywords: *Lignocellulosic waste, bioenergy, microbial fuel cells (MFCs), electricigens*

INTRODUCTION

Sufficient energy generation (in any form) is a boon for countries to survive in the population explosion responsible for the depletion of the existing energy sources around the world. Due to no alternative measures, conventional energy sources will no longer exist; hence, alternative non-conventional sources are being explored worldwide. A huge increment (approx. 200%) of total energy in the last two decades was seen due to the explosion of the human population worldwide [1]. Novel biotechnological approaches are in progress and explored through alternative approaches towards energy production, which could minimise the load on conventional energy sources, thus maintaining a balanced ecosystem [2]-[3].

There is a global fascination with estimating and implementing alternative energy sources. Harnessing energy from inorganic or organic waste can offer an efficient solution for simultaneously addressing energy and environmental challenges. Through microorganism metabolism, MFCs can directly convert chemical energy stored in many bio-sources of degradable organic matter to electrical energy [4]-[5]. H_2O and CO_2 are being achieved as clean by-products, and the renewable exo-electrogens support MFCs with the great potential to become a clean green energy source. MFCs are recommendable over conventional methods (e.g., flocculation/froth flotation) used for wastewater treatment due to the utilisation of microbial metabolic activities for energy production without high energy input and environment-friendly [6]. Also, in the conventional wastewater treatment industry, some of the significant challenges are the operational cost (around 3% of the world's electricity is consumed) and sludge disposal costs (50% of wastewater treatment) [7]-[8].

Other factors, such as ineffective treatment of the produced Greenhouse gases (GHGs) and dissolved substances like ammonia and phosphates, are another technical challenge in conventional wastewater treatment. The scientific community, over the years, has shown interest in extracting energy from biomasses, mainly from cellulosic biomasses, such as bioethanol production [1],[9]-[10]. Cellulosic material (with lignin and hemicellulose) is widely distributed over the terrestrial earth in the form of plant products, agricultural residues, and woody biomass. This most abundant

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polysaccharide material is a good source of a tremendous quantity of renewable bio-resources with varied applications [11]-[14].

However, the yield of bioethanol from cellulosic biomass depends on the effective pre-treatment processes (physical, chemical, physio-chemical, or biological) as well as the type of substrate used [12]. The conversion of a cellulose molecule (a dimer) into a simpler glucose molecule (a monomer) is a technical hindrance that needs to be achieved appropriately. Nevertheless, using MFCs, a bio-electrochemical concept and a promising technology, the breakdown of cellulose molecules to generate electrical energy can be achieved directly [4]-[5],[15]-[16].

Further, bioenergy generation from lignocellulosic waste is a promising area proliferating worldwide that can help improve a country's economic development. Several countries are working on energy generation (both biofuels and electricity) from the available natural resources [2],[14],[17]. Countries like the USA, China, Brazil, Canada, and India are promoting the utilisation of biomass residues for bioenergy production. As per the International Energy Agency (IEA), biofuel production expanded by 6% in 2019 and possibly 3% annual production is expected in the next 5 years. Again, the consumption of transport biofuel is expected to almost triple (till the year 2030) up to 298 Mtoe (Millions of tonnes of oil equivalent) with the sustainable development scenario (SDS) based on Figures 1a and 1b [18].

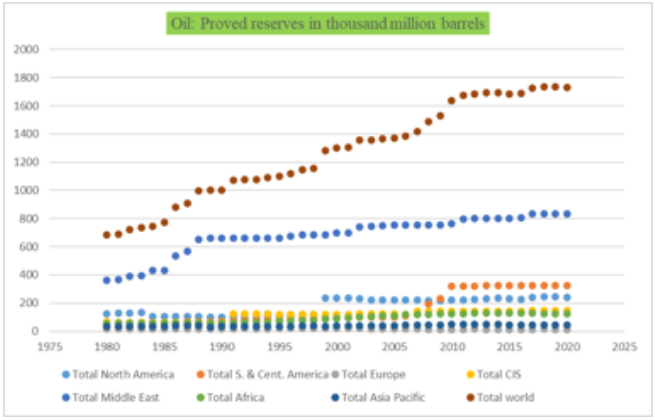


Figure 1 (a) Oil proved reserves in thousand million barrels [19]

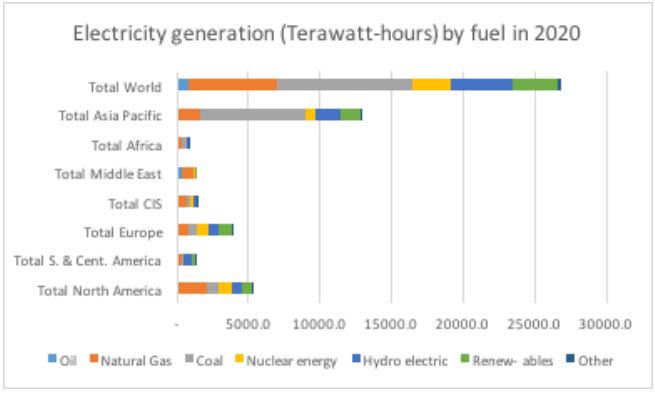


Figure 1 (b) Electricity generation (Terawatt-hours) using different sources of fuel in 2020 [20]

However, IEA is concerned about the slow global biofuel production for various reasons. In the global bioenergy sector, in 2019, there was an increase in power generation of up to 5% since 2011. At the same time, global electricity generation from bioenergy is expected to increase by 6% annually through 2030, based on Figures 2a and 2b [21].

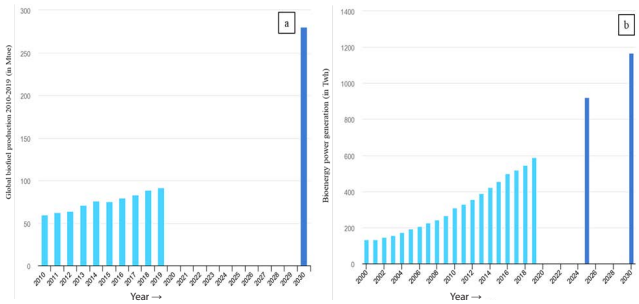


Figure 2 (a) Global biofuel production 2010-2019 compared to consumption in the SDS and (b) Global Bioenergy power generation in the SDS, 2000-2030 [22]

In India, there is a vast potential for renewable energy generation from sources like solar, wind, biomass, cogeneration bagasse and small hydro. Among the total potential of renewable energy generated in India, the potential energy generated from the solar sector is approx. 68% (748990 MW), wind power potential approx. 28% (302251 MW), bagasse-based cogeneration in sugar mills contributes approx. 0.46% (5000 MW), biomass power 1.0% (17,536 MW), and from waste to energy is approx. 0.23% (2554 MW) (Figure 3) [23].

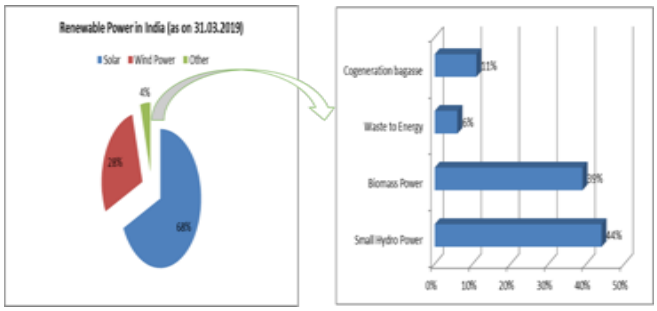


Figure 3 Estimated renewable power in India as of 31.03.2019 [24]

The generation of electricity through MFCs, a bio-electrochemical device, has gained promising importance and yet remains a technical challenge. These bio-electrochemical devices, with the use of microorganisms as a catalyst, facilitate the conversion (through oxidation) of chemical energy of organic matter (stored in organic matter at the time of photosynthesis) and of inorganic matter into electrical energy [15],[25]-[27]. Two primary types of biological fuel cells are available for low-power devices: enzymatic (EFC) and MFC. It has been seen that, in terms of life span, MFCs have a leading edge over EFCs despite the fact that both systems are catalyst-dependent. However, EFC catalysts are substrate selective, but MFC catalysts are not substrate dependent. MFCs system can be supplied with several substrates such as wastewater, biomass, landfill leachate, sludge to sediments and any liquid rich in carbon content [28]-[29]. Due to their capacity

to convert chemical energy into bioelectricity, MFCs have a wide range of applications, including powering clocks, mobile phones, pumps, and biosensors [17].

Additionally, MFCs can serve as in-situ power sources for operating small electrical devices like biosensors in remote locations where regular system maintenance and battery replacement are impractical, such as oceans and deep waters [29]-[30]. In contrast to a conventional energy source, MFCs have advantages like simplicity, high conversion efficiency, extensive operating conditions, and an eco-friendly approach. MFCs is not only limited to bioelectricity generation but to a wide range of applications like denitrification, dye degradation, wastewater treatment, phenol removal, chemicals production, CO_2 reduction, sensors, and robotics [5],[8],[31]-[32]. However, the operation cost and installation of the MFCs is high. Therefore, using cheap and abundant materials such as lignocellulose as the fuel can reduce the cost of the MFCs operation. Further, due to the recalcitrant nature of the lignocellulose biomass, this offers a challenge to use as substrates directly in the MFCs [14],[26],[32]-[33]. Therefore, the pre-treatment process of these biomasses becomes important and crucial for the effective utilisation in high yield of high-yield electricity generation.

Microorganisms have the potential to break various biodegradable organic compounds into water, CO_2 and energy [1],[26]-[27]. Among several hydrolysis procedures, like physical, chemical, physio-chemical, and microbial hydrolysis has advantages such as easy operating conditions, maximum rate of hydrolysis and, most importantly, an eco-friendly approach [34]. Among several microorganisms, *Pseudomonas, Clostridium thermocellum, Lactobacillus plantarum*, and *Cyanobacteria* are some of the good cellulolytic microorganisms [16],[32],[35]-[36]. MFCs system uses cellulolytic organisms (cellulose-degrading organisms), incorporating exogenous cellulases to generate electricity. Potter [37], an English Botanist, first reported the potentiality of bacteria to generate electricity from the decomposition of organic compounds [38]. The microorganisms serve as the linking bridge between chemical energy (substrate compounds) and electric energy. The main objective of this review paper is to explore and highlight the crucial role of lignocellulosic biomass in sustainable energy production, with a specific focus on two primary categories of fuel cells: indirect biomass fuel cells (IDBFC) and direct biomass fuel cells (DBFC). The paper aims to provide insights into the challenges and opportunities associated with these fuel cell technologies, emphasising their significance in the context of renewable energy. It also highlights the pivotal factors influencing the performance of MFCs, especially concerning efficient biomass hydrolysis and the role of electricigens in electron transfer mechanisms. Lastly, the paper delves into the importance of nutrient supplements for enhancing bio-methane production in anaerobic digestion processes, aiming to contribute to the broader understanding of sustainable energy production.

LIGNOCELLULOSIC BIOMASS ENERGY FUEL CELL

Lignocellulosic biomass energy fuel cells can be of two major types: (a) IDBFC are operated at high temperatures, whereas MFCs are operated at low temperatures. IDBFC uses direct carbon fuel cells (DCFC) and solid oxide fuel cells (SOFC) in the operation. Further, IDBFC technology requires pre-converting biomass into simpler molecules, such as sugars (e.g., glucose and xylose), biogas, syngas, or biochar, as usable fuels for subsequent electricity generation and (b) DBFC is a naïve electricity generation technology, where pre-conversion of biomasses into simpler usable substrates is not required [39]-[41]. However, limited research is being carried out by using naïve DBFC technology [42]. Lignocellulosic biomass is a complex polysaccharide molecule consisting of cellulose, lignin (non-carbohydrate molecule) and hemicellulose, one of the most abundantly available and renewal components in nature with numerous applications such as bioethanol production, electricity generation and many more [1],[17],[43]. In lignocellulosic compounds, C, H, O, P, N and S are major elemental constituents, whereas C, H and O primarily come from cellulose, lignin, and hemicelluloses, three major components of lignocelluloses biomass. P, N and S are present in minor components (such as protein) [40].

Cellulose $(C_6H_{10}O_5)n$, with high molecular weight (200,000 to 2,000,000 per residue) of a complex polysaccharide, is linked with D-glucose units (linked with β-1,4 glycosidic bonds) in linear arrangements [11]. Lignin is responsible for the mechanical strength of the plants since the high cross-linking of ether and carbon-carbon bonds (β-β, β-O-4 and β-5 bonds) that polymerise 4-hydroxy phenylpropanoid monomers (formed due to plant laccases and/or peroxidases) [44]. Lignin hindrance towards enzymatic accessibility during acidic pre-treatment occurs mainly through the simultaneous depolymerisation and repolymerisation reactions. Firstly, acid-promoted dehydration of Cα-OH in β-O-4 structure led to the formation of carbocation (a molecule in which a carbon atom has a positive charge and three bonds) Cα position of the lignin side chain [45]-[49]. Next, the formed carbocation is repolymerised with the nucleophilic sites of the aromatic ring with G or S structures (adjacent or another aromatic ring), leading to the generation of stable C-C bonds and thus increasing the molecular weight of the residual lignin, leading to lowering the enzymatic accessibility [49]-[50]. During depolymerisation and repolymerisation reactions, in the β-O-4 structure of polysaccharide molecule, the loss of hydrophilic functional groups such as the Cα-OH and formation of aromatic rings (condensed) intensify the undesirable hydrophobic interactions between lignin and enzymes, thus lowering the enzymatic accessibility [48]-[49]. Hemicelluloses, present in the primary and secondary cell walls of plants, is a heterogenous biopolymer consisting of mannans, glucomannans, xylans, xyloglucans and β- (1,3, 1,4)- glucans (mainly found in terrestrial plants) is linked through β-1,4 backbones [22],[51]. Due to its recalcitrant nature and complexity, which hinders them from breaking down into favourable by-products, the lignocellulosic biomass cannot be efficiently hydrolysed (lignocellulolytic activity) directly by microorganisms.

Lignohemicellulosic wastes are of natural origin and possess biochemical properties ensuring quick microbial decomposition at comparatively normal operating conditions. Additives are generally used to mitigate lignin inhibition and are significant

for sustainable lignocellulosic biorefinery. Despite the physical, chemical, or physio-chemical approach known for the pre-treatment of lignohemicellulosic biomass, the biological approach to sugar release from biomass (lignohemicellulosic biomass) offers high yields; however, the quantity of enzymes needed for conversion with high yields is also high and remains a primary obstacle towards bioenergy (cellulosic ethanol/Bio-methane) commercialisation [1],[5]. Again, the application of additives, a critical interface between enzyme accessibility and activity, like between non-ionic surfactants and non-catalytic proteins, has been considered a promising alternative to boost the enzymatic hydrolysis yields [52]. The inefficient hydrolysis of biomasses such as lignocellulosic biomass in the anode chamber leads to a low power density (PD) in MFCs. The main technical obstacle lies in the recalcitrance of lignocelluloses to fermentable sugars. Particularly, the hierarchical structure of biomass hinders the release of fermentable sugars through chemical or enzymatic means [14],[17]. Crystalline cellulose, characterised by a highly ordered and stable hydrogen bonding structure, poses difficulties in permeation. In order to overcome these limitations, pre-treatment processes are necessary to liberate various reducing sugars. However, the effectiveness of pre-treatment depends on biomass characterisation, which is a crucial factor for maximising product recovery in the MFC biorefinery process [17],[33].

Additives in Saccharification of Biomass

Exploration of microorganisms in an optimised manner can produce a wide range of liquid and gaseous fuels such as methane, bio-methanol, bioethanol, biodiesel, and biohydrogen. MFCs is a type of system that can use microbial machinery to generate fuels (liquid and gaseous) through the conversion of organic substrates. Additives are known to improve the quality of pellet of biomass efficiently; however, there is continuous research being carried out for a cost-effective one that could enhance the properties (physical and chemical) of the raw biomass as well as decrease the friction during the pelleting process for low energy consumption [53]-[54]. Pelleting is a process through which finely ground mesh (simpler polysaccharide biomass) can be converted into compact, high-energy density biomass in the form of free-flowing pellets or capsules. Pre-treatment of raw biomass is necessary before proceeding to pelleting to achieve high energy consumption of pelleting and low product calorific value of pellets [54]. Rosin et al. [53] used several biomasses such as Molasses, HSC-residue, montan resin, ash, slaked and starch (0-1 mm size and at 5%) to improve the pellets' density.

Proteins and synthetic polymers act as effective additives in saccharification of corn cobs, sugarcane bagasse, wheat straw and pretreated softwood [55]. Other additives, such as paraffin and crude glycerin, are of high quality and efficient additives. Paraffin is a colourless, white mixture made from crude petroleum. Crude glycerin is a significant by-product during biodiesel production and consists of an abundant impurity that inhibits its direct usage in industries [56]. The calorific value of crude glycerin is approx. 27 MJ/kg [53],[57]. Besides being a high-quality and efficient additive, crude glycerin cannot be efficiently utilised due to technical restrictions, as it could lead to environmental pollution [54].

As per Zhou et al. [58], lignosulfonate (LS), a strong hydrophilic nature surfactant, can result in the net enhancement of enzymatic saccharification of lignocellulose biomass. As the bound lignin (lignin remaining in solid substrate after pre-treatment) has a high affinity to cellulase from binding cellulase nonproductively, LS could act as a surfactant to block the bound lignin. The enhancement is proportional to the amount of LS applied [59]. Again, a non-ionic and amphoteric surfactant, Tween 20, has been found to be effective in the enhanced saccharification of biomass [60].

Several strategies have been conducted so far to improve the profitability of enzymatic hydrolysis and overcome challenges related to efficient hydrolysis. Along with the high yield, operational costs related to the yield of the enzymatic hydrolysis remain the focal objective to improve the economic feasibility of the process. Among these, the application of novel cellulolytic enzymes (from microorganisms) with better performance, protein engineering of the cellulase enzymes and the improvements in pre-treatment technologies remain the targeted strategy [4],[52]. Again, the application of additives, a key interface between enzyme accessibility and activity, like between non-ionic surfactants and non-catalytic proteins, was considered a promising alternative to boost the enzymatic hydrolysis yields [52].

Macro and Micro-Nutrients as Additives

Studies show that nutrient supplements (NS), both micro- and macro-nutrient supplements, can stimulate bio-methane production as well as improve process stability, and widely drawn the attention of researchers as an important topic for agro-industrial biogas mono-digestion plants [61]-[64]. Nutrient supplements improve the system's stability by maintaining the pH within optimum values (avoiding volatile fatty acids accumulation/ or providing a minimum alkalinity level) [62]. Methanogens (methane-producing microorganisms) are extremely dependent on micronutrients such as Fe, Zn, Ni, Cu, W, Co, Se, and Mn, which act as a crucial cofactor and can stimulate reactions catalysed by metalloenzymes (enzyme proteins containing metal ions/metal cofactors), ferroxins, and formation of cytochromes [63],[65]. Macronutrients (C, S, P, N, H) play a promising role as a buffering agent in the growth and metabolism of anaerobic microorganisms and in several enzymatic reactions in the biochemical pathway of methane formation. Ni, Co and Fe are essential cofactors of carbon monoxide dehydrogenase, methyl-H4SPT: HS-CoM methyltransferase, acetyl-CoA decarbonylase, methyl-CoM reductase and several other enzymes involved in the acetoclastic methanogenesis pathway, are most studied nutrients till date. Again, these metals also have a promising role in the acetotrophic pathway of methanogenesis (acetate oxidation to carbon dioxide and hydrogen followed by hydrogenotrophic methanogenesis) [62],[66].

Further, high concentrations of nutrients (macro and micro-nutrients) can lead to the inhibition of the anaerobic digestion (AD) process [62]. It has been observed that appropriate nutritional balance (macro and micronutrients) in the AD process results in controlled microbial generation time, degradation rate and gas production. If not, the performance of AD will deteriorate, resulting in poor

process stability and unsteady methane production [67]. Hence, it has been noticed that, as the AD process can only be carried out successfully with certain nutrient concentrations, the mere presence of the nutrients in the feedstock does not always ensure that AD is efficient [65]. Therefore, the determination of a suitable dose of nutrients remains a great challenge to the efficient performance of methane production from agricultural biomasses. An MFCs system utilises monosaccharide molecules or other low molecular weight compounds. According to Catal et al. [68], nearly all monosaccharides (obtained after hydrolysis of lignocellulosic biomass) are good sources for electricity generation. Therefore, a knowledge of the properties of biomass cell walls, along with the appropriate pre-treatment method, is very important for maximum electricity generation.

MFCS LAYOUT AND WORKING PRINCIPLE

Microbial Fuel Cells (MFCs)

MFCs comprise three major components: an aerobic cathode chamber, an anaerobic anode chamber, and a cation exchanger. Microorganisms in MFCs interact with electrodes via electrons delivered through an electrical circuit. In an MFCs chamber, the anode serves as storage of growth media, a mediator (no mediator in mediator-less MFCs), a microorganism and an electrode (i.e., anode). The anode is known as the growth compartment for suitable microorganisms, converts the fuels into CO_2 protons and generates electricity through metabolic reactions [40]. Further, metabolic reactions are stopped before completion, and electrons (intermediate) are extracted to generate electricity [69]. Electrical power generation occurs due to the potential difference between the anode and cathode [42],[70]. Based on how the transfer of electrons takes place from bacteria to the electrode (anode), MFCs can be categorised into two types: (a) mediator MFCs, in which mediators (or electron shuttles) are included in the system and (b) mediator-less MFCs, where mediators are not included in the system [71].

The chemical process involved in MFCs technology is as follows:

$$\text{At anode:} \quad C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H+ + 24e- \qquad (1)$$

$$\text{At cathode:} \quad 6O_2 + 24H+ + 24e- \rightarrow 12H_2O \qquad (2)$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Electric Energy} \qquad (3)$$

MFCs, with the help of microorganisms, utilise various substrates such as chitin, cellulose, acetate, lactate, butyrate, ethanol, and glucose, as from other sources like aquatic sediment organic water, domestic wastewater, paper recycled wastewater and food industry wastewater, and generate electricity [68]. The microorganisms serve as the 'linking bridge' between the chemical energy stored in the substrate and electric energy. To date, thorough research is going on the effective utilisation of lignocellulosic biomass for high electricity generation through MFCs technology. Since the substrate is a source of nutrients and energy, the selection of substrate is very important for any biological process (Figure 4).

The amount of electricity generation is dependent on several factors, such as the rate of substrate degradation, the circuit resistance, the rate of electron transfers from the bacteria to the anode, the proton mass transfer in the liquid, and the performance of the electrode and the external operating conditions [39],[42],[70],[72]-[73].

Furthermore, electricity generation is also influenced by electrode materials used in MFCs systems. This is due to the fact that the electrode materials vary in their properties (physical and chemical), such as area of the surface, electric conductivity, and chemical stability. Hence, they have an impact on the attachment of the microbes, transfer of electrons, electrode resistance and the rate of electrode surface reaction [38]. Therefore, for the high performance of MFCs, a suitable selection of electrode materials is important.

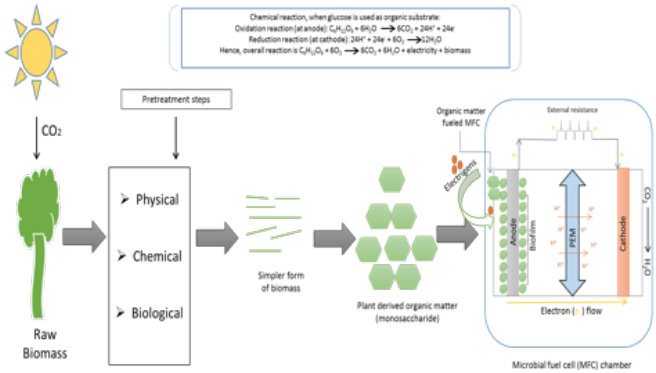


Figure 4 Basic principle and workflow diagram of a microbial fuel cell (MFC) chamber with biomass as organic substrate

ELECTRICIGENS

In 1911, Potter [37] introduced the use of microorganisms in MFCs for the first time. The choice of substrates applied in MFC affects the composition of the bacterial community at the anode. Again, at the same time, it influences their performance in terms of power density (PD) and Columbic efficiency (CE) [74]. Simple carbohydrates such as sucrose, glucose, fructose, xylose, trehalose, and maltose are more feasible for an effective MFC system. Several studies have also been carried out using composite substrates (such as cellulose, starch, pectin, molasses, and chitin) that were explored for bioenergy generation in MFC systems [74]-[76].

In MFCs technology, microorganisms used to oxidise the organic compounds and transfer the electrons via an anode chamber are termed as 'electricigens', and suitable electricigens play a key role in the effective operation of MFCs. The performance of MFCs is significantly affected by the availability of Electricigen, which acts as a key limiting factor [70]. Microorganisms utilised in MFCs can be broadly classified into two categories: the first category consists of "conductive" electrons, while the second category comprises non-conductive types that rely on natural or artificial mediators to generate power [29]. The perception of the theory of electricity generation by microorganisms is >100 years old, and the idea of MFC technology for electricity production has been thoroughly studied for the last 50 years [4],[77].

Table 1 List of some common electricigens used in MFCs [17],[30],[53],[61],[69]

Sl. No.	Microorganism	Gram positive/ Gram negative	Substrate used
1	*Actinobacillus succinogenes*	Gram negative	Glucose
2	*Aeromonas hydrophila*	Gram negative	Acetate
3	*Gluconobacter oxydans*	Gram negative	Glucose
4	*Alcaligenes faecalis*	Gram negative	Glucose
5	*Streptococcus lactis*	Gram positive	Glucose
6	*Clostridium beijerinckii*	Gram positive	Starch, glucose, lactate, molasses
7	*Clostridium butyricum*	Gram positive	Starch, glucose, lactate, molasses
8	*Escherichia coli*	Gram negative	Glucose, sucrose
9	*Pseudomonas aeruginosa*	Gram negative	Glucose
10	*Proteus mirabilis*	Gram negative	Glucose
11	*Enterococcus gallinarum*	Gram positive	Glucose
12	*Erwinia dissolven*	Gram negative	Glucose
13	*Desulfovibrio desulfuricans*	Gram negative	Sucrose
14	*Acetobacter aceti*	Gram negative	Glucose
15	*Geothrix fermentans*	Gram negative	-
16	*Acidobacteria capsulatum*	Gram negative	-
17	*Enterococcus faecalis*	Gram positive	-
18	*Rhodoferax ferrireducens*	Gram negative	Glucose
19	*Shewanella sp.*	Gram negative	-
20	*Sulphur-reducing bacteria (SRB)*	Gram negative	-
21	*γ-Proteobacteria*	Gram negative	-

Electricigen used in the anodic chamber of MFCs system is one of the primary factors for an efficient electricity generation performance. Several types of microbes have been used in an MFCs system, as listed in Table 1. Coupling the linkage of electricigens to the electrode substrate via electron transport is a major challenge in generating electricity in the MFCs system. Further, factors like growth characteristics, species distribution, metabolic characteristics, and the electricity mechanism of electricigens play a vital role in the development of MFCs [79]-[80].

Electricigen's Electron Transfer Mechanism

The process of electron transfer is basically carried out in two stages: (1) Inside to the outside of the cell membrane and (2) extracellular electron transfers, respectively [79].

Inside to the Outside of the Cell Membrane

Electron transfer in electricigens from inside the cell to the extracellular environment occurs via two processes, (i) from cytoplasm membrane to outer membrane cell and (ii) from outer membrane to electron receptor cell. Electricigens, such as *Geobacter* (gram-negative), *R. ferrireducen* (gram-negative), *Shewanella* (gram-negative), *Enterococcus gallinarum, Clostridium butyricum EG3, Pelotomaculum thermopropionicum, Shewanella oneidensis, Pseudomonas aeruginosa, Geobacter sulfurreducens* and *Acidithiobacillus ferrooxidans, Methanothermobacter thermautotrophicus, Brevibacilus agri (firmicutes), Clostridium acetobutylicum*, and *Clostridium thermohydrosulfuricum* can oxidise organic compounds (like sugar) to by generating reducing power (NADH) on its membrane and transfer the free electrons to the electron receptor on the membrane [70],[79],[83]-[84].Furthermore, certain species, such as *Escherichia coli*, can produce hydrogen sulfide when exposed to cysteine or glutathione, and they demonstrate good performance in MFCs at ambient conditions. Additionally, the utilisation of anodes featuring "nano spikes" seems to enhance the susceptibility of several bacterial species to release electrons more readily [29]. Several studies have shown that gram-negative bacteria, as compared to Gram-positive bacteria, have lower Zeta potential (negative surface charge), and due to these differences in cell surface charges, their electrogenic capability also varies [83]-[84]. Gram-positive bacteria (thicker cell wall, 10-80 nm) lack an outer membrane as compared to gram-negative bacteria and may be encased in a glycoprotein S layer. The chemical composition of Gram-positive bacteria comprises 90% peptidoglycan and 10% teichoic acid. Further, covalent bond formation between peptidoglycan and teichoic acid may result in higher surface Zeta potential [83]. On the other side, with components like peptidoglycan (approx. 10%) and with lipopolysaccharide, lipophosphate and lipoprotein (constitutes outer membrane), outer membrane protein and periplasm, gram-negative bacteria have lower Zeta potential (negative surface charge). Further, several other components of the electron transport system, like NADH dehydrogenase, flavine enzyme, quinones, and cytochrome C on the cell membrane, play a vital role in the electron transfer process from the cell to the extracellular medium.

Electron Transfer on Extracellular Membrane

Electron transfer on the extracellular membrane may take place via two (2) routes: (a) direct electron transfer and (b) indirect electron transfer mechanisms.

Direct Electron Transfer

Despite the complex, not fully clarified mechanism of electron transfers in MFCs, several pathways for the electron transfer from electricigens to electrodes have been proposed in studies. It is obvious that the process of electron transfer through electricigens is not a natural phenomenon. In direct electron transfer (DET) or mediator-less MFCs, microbes do not require any mediators for the transfer of electrons to an anode during respiration ("anode-respiring") [4],[20],[85]-[86]. For direct electron transfer, physical contact between the outer membrane and the anode is required, and an electron reaches the cell's outer membrane. MFCs electricigens form biofilms or electrically conductive nanowires

(pili and flagella) on the anode surface. Without any mediators (diffusional electrons) in MFCs, electron transfer takes place through the outer membrane cytochrome and nanowires or trans-membrane electron transport proteins by direct contact [79]-[80],[83]. In DET, electron transport proteins (ETP) have an important role as they transfer electrons from the cytoplasm to the outer membrane and finally to the anode. It has been found that, for efficient current generation in MFCs, direct electron transfer is the first choice. However, as the active sites of electron transport proteins are typically buried within the proteins, it results in a poor efficient electron transfer rate [4]. Microorganisms such as *Pseudomonas aeruginosa, Rhodoferax ferrireducens, Clostridium butyrium, Shewanella,* and *Aeromonas hydrophila* have been reported to oxidise organic matter by donating electrons directly to the anode to complete their metabolism process [20], [83].

In the case of mixed culture (like in a bacterial consortium), exoelectrogens use a direct interspecies electron transfer (DIET) mechanism for intermediary electron transfer. Transcriptomic and genetic studies have markedly stated that a syntrophic association between *G. metallireducens* and *G. sulfurreducens* (metabolising ethanol) produces conductive aggregates and exhibits the exchange of electrons [9],[87]. A syntrophic association between *Proteobacteria* and *Firmicutes* studied through community analysis reveals that Clostridium was involved in fermentation and ferredoxin-mediated transfer of electrons to the electrode. *Aeromonas, Desulfovibrio*, and *Tetrathiobacter* are sulfate-reducing-sulfur-oxidising bacteria (SRB-SOB) that directly transfer electrons to the electrode [12],[84]. In their study, Liu et al. [39] made an important observation regarding the Direct Interspecies Electron Transfer (DIET) phenomenon in co-cultures of *Geobacter metallireducens* and *Geobacter sulfurreducens*. They proposed that electron transfer through DIET is not limited to within the same species but can also occur between different species by utilising conductive materials as biological electrical connections [9].

Indirect electron transfer

Indirect electron transfer (IET) or mediated electron transfer (MET) involves the extracellular electron transfer through endogenous redox mediator (soluble). In this, the mediator enters the cell membrane (be reduced) and exists in the reduced state [78]-[79]. Some of the mediators used in IET are as follows: riboflavin, phenazine, emodin, melanin, quinones, and melanin. The reduced mediator transfers the captured electrons to an electrode (at the anode), producing an electric current and re-oxidises the mediator in its original status [79]. However, as the IET requires regular addition of exogenous redox mediators, this type of technology is technologically unfeasible and environmentally unacceptable.

EFFICIENT BIOMASS FOR MFCS TECHNOLOGY

Studies have also shown that sludge can be used as a good source of bacterial community to generate electricity in MFCs systems. Another advantage of using sludge is that it has a diverse mixed bacterial population, and reports suggest that the whole system's power output is enhanced in the case of a mixed population [88]-[89]. However, a disadvantage is that the mechanism and role of individual microorganisms in contributing to power generation cannot be fully identified. Both pure and mixed cultures have been explored to inoculate an MFCs system in generating electricity. However, as the cost of pure culture is high, it is less suitable for practical applications, such as in the case of treatment of industrial effluents. Electrogenic bacteria (like *Shewanella Clostridium butyricum EG3*) in mixed cultures (like soil and wastewater) are cost-effective as inoculate in a MFCs system, while in mixed cultures, non-electrogenic bacteria (such as methanogenic bacteria and denitrifying bacteria) though consumes, organic substrates but does not produce electricity [88]-[89].

It is well known that biomass can be used as a fuel source for energy production in MFCs. There are basically two types of biomasses, like lignocellulosic and non-lignocellulosic. The lignocellulosic (comprised of cellulose, hemicellulose, and lignin) biomass (such as sugarcane bagasse, wood, rice straw, rice husk, and corn cob etc.) also contains lipids, starch, proteins, minerals, and inorganics [1],[73]. Examples of non-lignocellulosic include algae, sewage sludge, and animal manure. At the same time, it is very important to identify potential truly waste biomasses because not all crop residues can be considered as waste. This categorisation depends upon the decomposition of the materials, which is very significant for the long-term sustainability of ecosystems [2]. Literature study shows that biomasses such as algal biomass, wheat straw hydrolysate, rice straw, corn stove, corn stalk biomass, orange peels, lemon peel, solid potato wastes, chitin, kitchen waste, and cow dung have been explored and utilised as a fuel source in MFCs system [2],[17]. However, several factors, such as moisture content, bulk density, size and shape, and the amount of energy produced, influence the bioconversion process to determine the quality of biomass in MFCs.

LIMITATION AND IMPROVEMENT AREAS IN A MFCS SYSTEM

The specific metabolic processes in MFCs depend on the type of biomass/organic wastes and the operational conditions of the MFCs. Numerous factors influence the performance and energy yield efficiency of MFC technology. Among these, key considerations include the composition of the feedstock, availability of electron donors and acceptors, pH levels of the anolyte and catholyte, temperature, and the materials used for the anode, cathode, and membrane [29],[73],[90].

pH plays a crucial role in MFCs as it impacts both the activities of anodic microorganisms and cathodic reactions. The accumulation of protons leads to acidification of the anolyte, while the cathode chamber experiences electrolyte alkalisation. The use of pH buffers like phosphate or bicarbonate (pH 7.0) has been proposed to control the pH of the anolyte. However, employing such buffers may lead to increased operating costs and impose additional burdens on effluent desalination or phosphorus removal [70],[90]-[91]. The ideal pH for optimal performance in MFCs varies significantly based on the specific type of microorganisms involved. Zhang et al. [92] conducted a study to examine the impact of pH on MFC performance and the

composition of the anodic microbial community. The results indicated faster COD removal occurred under acidic pH conditions, with Simplicispira, Comamonas, Variovorax, and Acinetobacter identified as the predominant communities under such acid conditions.

It is well known that the amount of current production in an MFCs technology is proportional to the potentiality of a bacterium to utilise a substrate (through oxidation) and transfer electrons to the anode electrode. MFCs technology has been improving in recent years. Considering the point of large-scale wastewater treatment, the power generated by MFCs is very low. Sediment MFCs is the only technology used for practical applications that produce power from sediment by inserting an anode in sediment and linking it via an electrical circuit towards a cathode placed in the superimposing aerobic seawater [33],[93]. Also, there are challenges like turbulence in the compartment membrane resistance in the proton transportation process as a part of scale-up and practical application [94].

There are mainly two types of issues related to MFCs technology in power generation. Firstly, substrate concentrations have direct relationships with the power generation capacity in MFCs. More than a precise value of the substrate concentration, the power generation will be stopped [29],[33],[70],[94]. Secondly, the proton exchange membrane (PEM), which separates the anode and cathode chambers, is the main source of high internal resistance (Rin) in the two chambers of MFCs. As a result of high internal resistance (Rin) due to PEM, the MFCs output is restricted [94]. Biocathode is supposed to improve oxygen oxidation in MFCs, thus removing the requirement for catalysis by oxygen oxidation on the cathode. Again, the novelty in the design of MFCs by reducing internal resistance by removing PEM can improve power generation. In theory, any living and respiring cell known to humans has the potential to release electrons and be utilised in MFCs. However, generating enough energy from MFCs to power larger households or industrial appliances is not feasible. Nevertheless, MFCs can serve as reliable long-term power sources in remote or hazardous areas, offering advantages over conventional batteries due to their lower maintenance requirements and intervention needs [29],[95]-[96].

Another challenge for researchers involved in MFC technology is to transform laboratory-scale technology to field-scale. Parameters such as choice of biomass substrate (with low lignin content), electrochemical, physical, chemical, engineering, and other microbial parameters are crucial for MFC scale-up and field implementation. Chemical properties of the waste (biomass/organic waste) influence the organic loading rate (OLR), which has a major effect on anodic biofilm during the fermentation process. Production of acidic metabolites during fermentation can damage the anodic electrolyte. Hence, the efficiency of OLR-fueled MFCs should be carefully optimised. A rise or decrease in OLR has an ample impact on hydrolic retention time (HRT), chemical oxygen demand (COD) removal and current density affecting electron transport performance in the MFCs process [24],[97]. It is likely that given the continued interest and support for this research, advancement in this area may prove for other applications as well.

CONCLUSION

There is a wide variation in MFCs electricity generation, which, along with several factors, mainly depends upon the ability of electricigens. Also, the path of every electron transfer has its advantages as well as disadvantages. Lignocellulosic biomass-derived biohydrogen (electricity) is an emerging technology that can fulfil the demanding needs of humans with respect to non-renewable, depleting energy resources. A deeper study on electricigens and their electron transfer mechanism in an MFCs system will help to provide more improved approaches to enhance the performances of MFCs. Recent studies help to understand that stable electricity is achievable in an MFCs system using better-processed agricultural biomass residues, which is the most abundant component (i.e., cellulose) of plant biomass readily available as a waste material in many parts of the world.

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