Chapter 2

Literature Review

2.0 Literature Review

Microbial fuel cell (MFCs) is a technology that utilizes microorganisms as biocatalysts for oxidizing inorganic/organic matter and producing current. Electrogenic bacteria generate electrons at the anode surface, which travel across a load wire towards the cathode [38]. Another modification of the system is the application of enzymes instead of electrogenic microorganisms, known as enzymatic biofuel cells [39]. Studies found that MFCs that utilize mixed cultures achieve higher power densities than pure cultures [40], [41]. Multiple varieties of materials have been used in the construction of MFC, increasing diversity in the MFC configurations working under various parameters like at different temperatures, pH, electron surface areas, electron acceptor, duration of operation and reactor size [42]. MFC is an ideal method for generating renewable electricity from biomass. Biomass can include bio-wastes from complex organic sources such as human, animal and food processing wastewater [13], [43]. In some early studies, the addition of mediators/electron shuttles (for carrying electrons from cells to the electrode) was proven for a noteworthy increment in power density [44], [45]. Bacteria that can transfer extracellular electrons without mediators are known as exoelectrogen [38]. Some classes of Proteobacteria, Acidobacteria and Firmicutes are active in extracellular electron transfer [46]. Some of the electrogens have conductive appendages known as nanowires. These nanowire helps to build mediator-less MFCs, which are more beneficial than mediators containing MFCs [46]. MFC includes two electrodes, anode (negative terminal), and cathode (positive terminal) [47]. Electrodes are placed within single or dual-chambered MFC. Both chambers are separated by a proton exchange membrane [47]. Two-chamber MFC needs additional expense in terms of aeration for providing oxygen at the cathode [47]. The power density of MFCs is much less than other fuel cells. If we want to consider it as a commercial and economical way of power production, then it is highly needed to reduce the cost of MFCs

construction and operation. Utilizing wastewater to make this technique commercial, sustainable and economical is a further area of research in wastewater treatment.

2.1 History of Microbial fuel cell

M.C. Potter (1911), a botany professor at the University of Durham, founds that electric energy also generates during the microbial degradation of organic compounds [48]. Later, this concept was applied to harvest the new power source by constructing a primitive MFC in 1931 using *E. coli* [49]. In the early 1980s, M. J. Allen and H. Peter Bennetto studied fuel cells and explained MFC's mechanisms [50]. In 1990, Habermann and Pommer constructed the first MFC wastewater treatment system with a mixed microbial culture in activated sludge [51]. The electron conduction characteristics of the *Geobacter species* were found in 1994 [52]. The anodophillic property of *Geobacter* was discovered in 2003 [53]. MFC is currently the most needful area in research for bringing out its potential for power production by optimizing electrodes, microorganisms, mediators and proton exchange membranes.

2.2 Principle of MFC

In MFC, at the anodic compartment, the microbe utilizes chemical energy obtained from organic substrates via their cellular respiration pathways and transfers this energy into the form of electrons. In the anaerobic environment, carbohydrates are metabolized into protons, electrons and carbon dioxide [54]. Figure 2. 1 depicts the primary mechanism involved in the MFC operation.

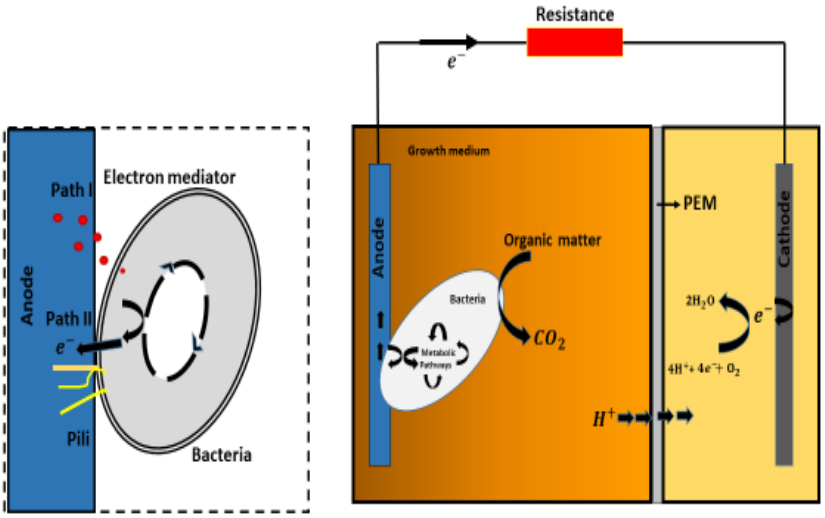

Figure 2. 1 The basic mechanism of microbial fuel cells

Hence electrons generated at the anode move toward the cathode via an external load. At the cathode, oxygen is reduced and generates water. The charge difference between these two electrodes generates bioelectricity. Chemical reactions that occur at both electrodes are as follows [55]:

Reaction at the anode:

$$C_{12}H_{22}O_{11} + 13\ H_2O \rightarrow 12\ CO_2 + 48e^- + 48H^+ \quad (2.1)$$

Reaction at the cathode:

$$6O_2 + 24e^- + 24H^+ \rightarrow 12H_2O \quad (2.2)$$

2.3 Material used in the MFC system

MFC configurations vary based on the MFC chamber, substrate, microorganism, electrode material, and membrane type. **Error! Reference source not found.** illustrates a general view of the essential components of MFCs.

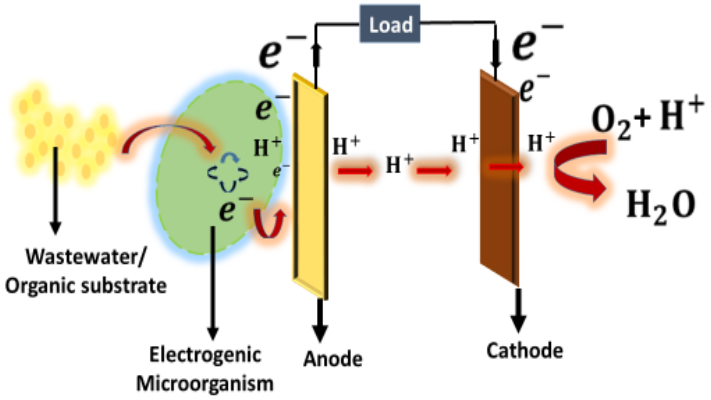

Figure 2. 2 Basic Components of microbial fuel cells

2.3.1. Anode

The selection of anode material is crucial for the efficient execution of MFC. Anode selection is a critical step that includes various parameters such as terminal electron acceptor, surface area, biofilm formation over the surface of the anode and their efficiency in terms of electrochemistry [56]. A considerable range of metal and carbon-based anode materials have been explored to improve the performance of MFC [56]. An anode material should be highly conductive, chemically stable, and have a large surface area with appropriate microbial adhesion properties. The widely used anode material is carbon-based materials. Among carbon-based materials, graphite variants (plates, rods, felt) and carbon variants (brush, cloth, mesh, viel, felt, paper, etc.) are widely used anode materials for MFCs [56] Metallic anode materials have an antimicrobial activity that makes bacterial growth impossible on the anode surface. However, a recent study demonstrates that electrogens could form colonies on the metal surface and a highly efficient electrochemically active biofilm on the metallic anode [57]. Metallic anodes are highly conductive such as copper, gold, nickel, titanium and silver [58].

2.3.2 Cathode

Carbon fiber, carbon paper, carbon cloth, graphite sheets, and graphite rods are widely used cathode materials in MFCs [59]. Carbon fiber fabric and carbon paper are stiff, brittle in texture and available as damp-proof and plain versions. Carbon cloth is more flexible and porous than carbon paper [59]. Compared to carbon cloths, carbon foam is a little thicker and has more space for microorganism growth, as reported in MFC research [60]. The most widely used form of graphite electrodes for MFC electrochemical research is graphite rod, which has defined surface area, low porosity, composition, and high conductivity [61]. Graphite sheets are available in various forms and thicknesses, such as soft rods, sheets, granules, and fiber brushes. Graphite sheets also have a much-defined flat surface suitable for biofilm formation. However, sheets are nonporous and hence less effective for power generation. It has been found that graphite sheets can introduce porosity by pouring them into the saline solution and making holes throughout the sheets to increase the surface area so that a thick biofilm can form over it and assist in more power generation [62]. Graphite granules are chunks of graphite as an electrode, like a pencil tip. It is helpful for both cathode and the anode in packed bed reactors [63]. Graphite fiber electrodes are used at the anodes as it has a large porous surface area. Industrial brush machines manufacture graphite fiber brushes and the core of the fibers is made up of non-corrosive metal [64].

2.3.3 Membranes

In a double chamber MFC configuration, an ion-selective membrane separates the anode and cathode into two different compartments and permits protons to transfer towards the cathode from the anode. Ion-selective membranes maintain the anaerobic environment of the anodic compartment by preventing oxygen diffusion and anode electrolyte migration toward the cathode. A membrane should have a good ability for proton exchange [65]. Proton exchange

membrane (PEM) is also known as the cation exchange membrane (CEM) when it allows other cations, such as Na^{+}, K^{+}, $NH4^{+}$, Ca^{++} and Mg^{++}, along with hydrogen ions transport [65]. The three major issues affecting the membrane's performance in a fuel cell are oxygen permeability, conductivity and membrane stability [65]. PEM is further categorized as a nonporous (dense) and porous exchange membrane [66]. Nafion, Ultrex, Zirfons, and Hyflons are widely used cation exchange membranes. Nafion has high proton conductivity as it has a negative sulfonate group(hydrophilic) associated with a fluorocarbon chain(hydrophobic), which allows proton transport from membrane pores [67].

2.3.4 Substrates

In MFC, various ranges of substrates have been used and all of them give different results in terms of power density or other parameters of power generation. Ranging from complex organic mixtures of compounds and pure substrate to wastewater from various sources.

2.3.4.1 Glucose

A study demonstrates MFC performance based on *Proteus vulgaris*, concluding its dependency on initial carbon compounds (Glucose, Galactose, Maltose, Sucrose, Trehalose) used as a substrate. Studies demonstrate the possibility of increasing MFC performance by changing the primary carbon source [68]. Glucose initiated cells in MFC run in a very short time compared with galactose [68]. Rabaey et al. (2003) obtained a maximum power density of 216 W/m^3 from a glucose fed MFC [69].

2.3.4.2 Acetate and Butyrate

Acetate is an extensively used substrate a carbon source for power generation in MFC. Acetate is inert to fermentation, methanogenesis and other microbial conversions. Acetate is also a product of some metabolic reactions [70]. Using acetate in a single-chambered MFC led to the maximum power density generation of 506 mW/m^2, which is 66% more than the power density

(305 mW/m^2) generated by butyrate [70]. A study compares the coulombic efficiency (CE) of MFC utilizing four substrates acetate, butyrate, glucose and propionate, where acetate produces coulombic efficiency of 72.3%. In contrast, butyrate, glucose, and propionate have coulombic efficiency of 43.0%, 15.0%, and 36.0% respectively [71].

2.3.4.3 Lignocellulose

As lignocellulosic biomass is widely available in agricultural waste to be used as a substrate for cost-effective power generation, the only problem is that lignocellulosic biomass cannot be metabolized directly by the microbes. Proper utilization of lignocellulosic material requires hydrolysis of biomass and then conversion of cellulose into simple carbohydrates. Another alternative to this pretreatment is utilizing such a microbe with cellulolytic activity and electrogens simultaneously [72]. Xylose are one of the components obtained from the hydrolysis of lignocellulosic biomass, and no efficient microbial species are available for xylose conversion for bioethanol bioproduction. In research, lignocellulosic degradation in MFC obtained a power density of 69 mW/m^2 [73].

2.3.4.4 Cellulose

Cellulose is the primary form of carbohydrate available as organic matter in agricultural, municipal, and industrial wastewater. Cellulose is widely available and cheap. Electrogenic microorganisms, which can perform anaerobic hydrolytic conversion of cellulose, can be used to utilize cellulose as a substrate in MFC directly. Authors obtain power density up to 0.055 W/m^2 by utilizing microorganisms present in cattle rumen and cellulose as a substrate [74]. Power density up to 0.153 W/m^2 is obtained by feeding carboxymethyl cellulose as a substrate in MFC [75].

2.3.4.5 Wastewater

The main aim of wastewater treatment processes is to remove or minimize the organic component load from water resources. Wastewater from agricultural and industrial effluent contains high organic matter rich in energy value [76]. Electrogenic microbes utilize complex organic compounds in wastewater and release electrons [77]. Table 2. 1 summarizes the role of waste biomass/wastewater as a substrate in MFC.

Table 2. 1 List of various industrial or domestic effluent used as substrates in MFCs

Industrial effluent	Cathode	Anode	Power density	COD removal (%)	Reference
Starch processing Unit	Air cathode	Carbon paper	239.4 mW/m^2	98%	[78]
Beer brewery industry	Air cathode	Carbon fiber	830 mW/m^3	91.7%-95.7%	[79]
Food processing industry	Carbon paper	Carbon paper	371±10 mW/m^2	95%	[80]
Meat processing unit	Toray Carbon paper	Toray Carbon paper	354±10 mW/m^2	87%	[81]
Urban wastewater	Graphite cylinder	Graphite cylinder	25 mW/m^2	-	[82]

Chocolate industry	Graphite rod	Graphite rod	-	75%	[83]
Paper recycling Unit	Graphite fiber brush	Graphite fiber	501±20 mW/m^2	-	[84]
Animal waste	Graphite Rod	Graphite Rod	31.92±4 mW/m^2	-	[85]
Fruit peel waste	Copper	Zinc	72 mW/cm^2	-	[22]
Food waste	Carbon cloth	Carbon cloth	5.6 W/m^3	-	[86]

2.4 Configuration of MFC

It is necessary to develop an MFC configuration that is cost-effective in the manufacturing process, affordable, easy to scale up, have high coulombic efficiencies and power density for a realistic approach to MFC in day-to-day life. Generally, single and dual-chamber MFCs configurations with different designs are applicable in recent research.

2.4.1 Single chamber MFC

Single-chamber MFCs are very simple in construction and easy to scale up as compared to double-chamber MFCs. Installing a proton exchange membrane in a single chamber MFC is unnecessary but could be used wherever needed [87], [88]. The cathode should either have PEM placed at one side of the chamber, allowing protons to diffuse via the porous air cathode surface and utilize atmospheric oxygen [87], [89]. A study on single-chambered MFC with an air cathode obtained lower coulombic efficiency than MFC with a PEM as oxygen diffuses

towards the anode [90]. Eliminating the PEM also cuts the expenditure on the fouling of the membrane and the construction of MFCs. At the same time, oxygen diffusion occurs towards the anode, causing fewer electrons available to recover as current [91].

2.4.2 Dual-chamber MFC

Various configurations are available in dual-chamber MFCs, such as H shape, U shape and Up-flow tubular MFCs. Dual chamber MFC has a cathode in one chamber and an anode in another. A cation-selective membrane or salt bridge separates these chambers, allowing movement of protons toward the cathode and avoiding oxygen diffusion toward anodes [92]–[95]. The dual-chamber MFC is the most utilized configuration for checking the electrogenic activity of microbes and optimizing materials due to their inexpensive design. However, they have been found difficult to scale up [47]. H-type MFC is elementary and cost-effective. Hence, it has been widely used to check basic parameters in MFC research. It is significantly less efficient in power density owing to high internal resistance [47], [95]. A different kind of dual-chamber MFC configuration is a mini construction called Mini-MFC, which has 1.2 cm^3 total volume, using graphite felt electrodes. Mini-MFC has a large surface area to volume ratio, which allows for gaining high power output [96]. Another design is an up-flow MFC, which operates in continuous flow mode, assisting with the up-flow anaerobic sludge blanket system that can achieve a maximum power density of 29.2 W/m^3. Comparatively, it is easy to scale up for practical applications [92], [94].

2.5 Electrogenic microorganisms and extracellular electron transfer pathways

The bioelectrochemical system (BES) is an evolving area for bio-energy generation and the reclamation of wastewater [97]. BESs are able to transform the chemical energy of organic waste into electricity. Nowadays, the researchers are focusing on microbial electrosynthesis. They are also analysing the link between electron transport and metabolic processes inside and

outside of microbial cells. Microorganisms which are able to transport electrons extracellularly are known as electrogens. BES performance hinges on the electrogenic capacity of electrogens [98]. Atkinson et al. (2022) envisioned electrogens as living electronic system. Electrogens have redox mediators, cytochromes and nanowires as charge-carrying wires. The deposition of electrogens on the surface of electrodes are analogous to storage of charge in capacitors. However, accumulation of electrogens as a current channel between source and drain electrodes act as transistors.

Microorganisms (autotrophic and heterotrophic) acquire energy via metabolic decomposition of organic compounds such as glucose, sucrose and cellulose by cellular respiration, fermentation and photosynthesis [65] – [68]. A vast range of microbes (archaea, bacteria, cyanobacteria and specific eukaryotes) utilize extracellular mineral/metal (iron, manganese) as an electron sink during respiration. These microbes contribute electrons in extracellular electron transfer (EET) routinely [104]. EET is the key player that links electrodes (solid-state electron acceptors/donors) with the microbes. The depletion of oxygen (soluble electron acceptor) inside the electrochemically active bacteria leads to the transport of metabolically produced electrons outside the cell membrane [105]. The concept of the BES was initially proposed by Potter (1911). Various mechanisms have been proposed for EET in microbes [106], [107]. Under anaerobic conditions, metal-reducing bacteria such as *Shewanella oneidensis* MR-1 and *Geobacter metallireducens* oxidize organic substrate and transfer electrons to the metal (Fe (III) or Mn (III)) for completing the respiration cycle. Metal oxidizing microbes such as *Sideroxydans lithotrophicus* and Rhodopseudomonas *palustris* TIE-1 use metal ions such as Fe (II) as electrons to reduce O_2, CO_2 and NO_3^- [107].

2.5.1 Electroactive Biofilms

In BES, electrogens and their biofilm play a major role in transferring electrons from cell membrane to anode for the bioelectricity generation [108]. Microbial biofilms act as powerhouse of the BES. Tapia et al. (2009) speculated ample cell adhesiveness and electrons transport through the extracellular polymeric substances (EPS) layered carbon surface. Microbial attachment and biofilm formation over an anode surface are essential for the efficient functioning of BESs [110], [111]. In BESs, microbes develop a biofilm on the surface of electrode which interacts with metals for the exchange of electrons [112]. Water makes a primary portion (97%) of the biofilm matrix and the rest is made up of microbial cells and EPS [108], [113], [114]. A wide range of proteins, glycoproteins and glycolipids form a significant constituent of EPS [115], whereas a scarce amount of nucleic acids (extracellular DNA) are also present [116]. Linear and branched exopolysaccharides of size 500-2000 kDa are present in the EPS. Exopolysaccharides could be a homopolymer like cellulose and dextran or heteropolymer such as emulsion, alginate, xanthan and gellan [115]. Among the known bacterial EPS, Psl (neutral polysaccharide), Pel (cationic polysaccharide) and alginate have been mostly found to be active in biofilm formation. Reports state that in *Pseudomonas aeruginosa*, Psl polysaccharides play a pivotal role in the biofilm formation. As a scaffold, EPS anchors the cells together [104]. Hydrogen bonding helps in retaining 98-99% of the water in EPS to form a stable gel matrix, which hydrates microbial aggregates [108], [112]. The amount of EPS increases with time as biofilm gets aged [108], [110]. EPS has DNA (extracellular), which is vital for the preliminary attachment of bacterial biofilm with the microbe's surface and quorum-sensing control in order to release extracellular DNA [117]. The extremophile *Halanaerobium praevalens* was able to produce a more robust anodic biofilm in a hypersaline MFC by augmenting quorum sensing capabilities with the use of quinolone and external quorum sensing signals. There was a 95% increase in the quantity of biofilm. This led to 30%

surge in power density [118]. Proteins such as adhesins present in biofilm help in the molecular binding between electrode and biofilm [119]. Zhuang et al. (2022) developed an exopolysaccharides-deficient *G. sulfurreducens* in order to investigate the contribution of EPS in electroactive biofilm. The current generation capacity was reduced in the mutant biofilm as compared to the wild type because it expressed less EPS. As compared to the wild-type, the mutant *G. sulfurreducens* had a considerably lower EPS content, which led to a thinner biofilm and lower cell survival. Zhuang et al. (2022) also observed that *G. sulfurreducens* mutant with overexpressed pili developed a mature biofilm with prolonged time. This signified that EPS is necessary for rapid biofilm formation. However, it also indicated towards the role of pili in the biofilm formation. The mutant *G. sulfurreducens* had a lesser content of *c*-type cytochromes (*c*-Cyts) than wild-type *G. sulfurreducens*. Hence, EPS also assisted in anchoring of extracellular *c*-Cyt. This is crucial for EET in biofilms [120]. EPS is considered as a semiconductor and it has conductivity up to 10 to 10^3 μS cm^{-1} [106]. Confirmations were also obtained to support the semi-conductive nature of EPS. Lopes et al. (2003) showed semi-conductive features present in the form of conductive polymers/electrolytes in ionized/hydrated biopolymers and summarized the effect of ionic strength on the conductance capacity of the EPS (doping). Such doping processes enhance the conductivity of chitosan, starch and other saccharides [122]. Biofilm formation (Figure 2. 3) takes place in the following steps [123]:

(1) Brownian or chemotactic movement of microbes towards the electrode superficially,

(2) adhesion of bacteria to the surface of anode through flagella and pili,

(3) the emergence of microcolony and biofilm development on facets of the electrode (EPS and adhesins are generated for attachment while quorum sensing helps in biofilm formation) and

(4) dispersal of biofilm cells after maturity.

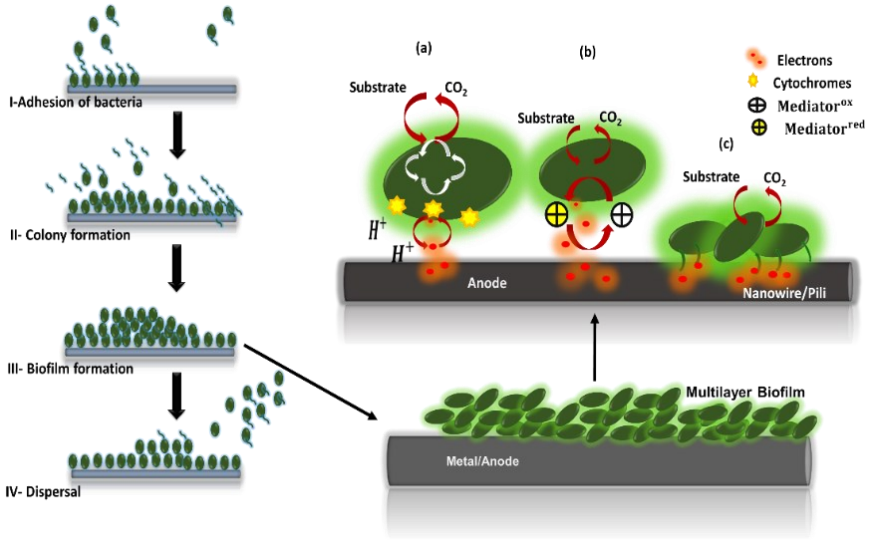

Figure 2. 3. Biofilm formation and electron transfer modes from bacteria to the anode

It has been reported that the non-polar hydrophobic surface of plastics and teflon attaches to microorganisms more rapidly than the hydrophilic surface of glass or metals [124]. The thickness of biofilm varies from species to species and the number of microorganisms. The biofilm thickness ranging from 15-30 µm was observed in pure culture biofilm of *Klebsiella pneumonia* and *P. aeruginosa*. In some *Geobacter* species have shown biofilm thickness of more than 40 µm [125]. In contrast, mixed culture (consortia) has reflected a biofilm thickness of approximately 100-200 µm [126]. Electrogens such as *S. oneidensis* and *G. metallireducens* transfer their electrons from the cell membrane to the anode through the following routes [127]:

(1) indirect exchange of electrons via electron shuttle

(2) via cellular appendages as nanowires and pili

(3) the direct transfer of electrons employing multi-heme c type cytochromes (redox proteins)

Microbes may adopt one or more than one way for EET. It has been observed that the combinations of these routes increase the rate of electron exchange [128]. For example, high current generation in microorganisms occurs by combining nanowires with cytochromes

existing in the exterior cell membrane of *S. oneidensis* MR-1 [129]. Direct EET-based membrane-bound redox enzymes or cytochromes, which get coupled with internal metabolic reactions, need a direct attachment between the cell membrane and the electrode (electron acceptors). Electrogens develop a conductive pilus known as microbial nanowire for the attachment [130]. Pili also helps in biofilm synthesis through cell aggregation and electron exchange in the biofilm [131]. It has been suggested that application of selective pressure to microbes promotes nanowire growth on the cell surface and leads to enhanced electron transfer to the anode [132]. Selective pressures are considered as forces that drive evolution via natural selection. Some of the phenotypes are more favourable than others, depending on the external conditions. Only microbes present in the first monolayer of anode biofilm are directly connected with anode surface. However, direct electron transfer needs very close contact between anode and microbial outer membrane cytochrome for an efficient BES operation [132]. Altamura et al. (2017) attempted a new approach by synthesizing an artificial redox biofilm made up of a bioengineered chimeric protein, which showed self-assembling property and acted as an electron transporter in the conductive biofilm. Recently, it had been observed that multicellular filamentous microbes were able to transfer electrons across small distances (few centimetre) via cytochrome redox potential gradient [134] and made efficient use of biofilm and anode which were parted by a large distance [135]. Indirect transfer of EET relies on the electron shuttles (redox-active small molecules) such as flavins, quinones and phenazines secreted by electrogens to relay electrons on the surface of electrode [136]. A good redox mediator should be nontoxic for microbial growth. The redox potential of the mediator must on the one hand be positive enough to allow a sufficiently fast reduction by the microbial redox systems and on the other hand be as negative as possible to minimize the energy losses [137], [138]. The redox mediators can be either exogenous (artificial compound) or endogenous (naturally secreted by microbial cells themselves). Endogenous mediators are

usually metabolites produced by the cells, such as riboflavins, flavins, flavin mononucleotides, phenazines, pyocyanins and quinones [44]. *S. oneidensis* MR-1 secretes flavins and riboflavin that play a crucial role in electron transfer as an endogenous mediator [139], [140]. Removal of riboflavin from *S. oneidensis* MR-1 resulted in the reduction in electron transfer rate by 70% [139].

2.6 EET pathways by electrogens

The multi-heme c-type cytochromes play a major role in the extracellular electron exchange at the cell membrane's spanning. Multi-heme cytochromes are proteins containing heme groups that augment the rapid transfer of electrons through the following ways:

(1) construction of a charge transport pathway linking to the intracellular electron carriers such as quinones and NADH [141].

(2) direct or indirect electron transfer to anode [127]

(3) electro-biochemical signals significantly contribute to direct transmission of electrons to other microbial cells [127].

Metal-reducing microorganisms such as *S. oneidensis* MR-1 and *G. metallireducens* oxidize organic substrate and transfer metabolically produced electrons to the metal (Fe (II) or Mn (III)) to complete their respiration process [142]. Figure 2. 3 demonstrates electron transfer modes in microorganisms.

2.6.1 EET pathways in Gram-negative bacteria

2.6.1.1 Metal reducing pathway in *S. oneidensis* MR-1

Under anaerobic condition, *S. oneidensis* MR-1 (gram-negative) utilizes solid phase metals (electrodes) (such as Fe(III) and Mn (III/IV)) as terminal electron acceptor [142]. A metal-reducing pathway (Mtr Pathway) has been observed in *S. oneidensis* MR-1 and in some of the

strains. Mtr pathway contains protein components identified as multiheme c-type cytochromes (MHCs) [143]. Flavins and few multiheme c-type cytochromes (MHCs) such as CymA, Fcc3, STC, MtrD, MtrA, MtrE, MtrB, MtrC, MtrF and OmcA are involved in oxidizing the quinoles inside the cytoplasmic membrane followed by the transportation of electrons outside the cell to the plane of electrodes [143], [144]. Figure 2. 4 represents electron transfer pathways in *S. oneidensis* MR-1.

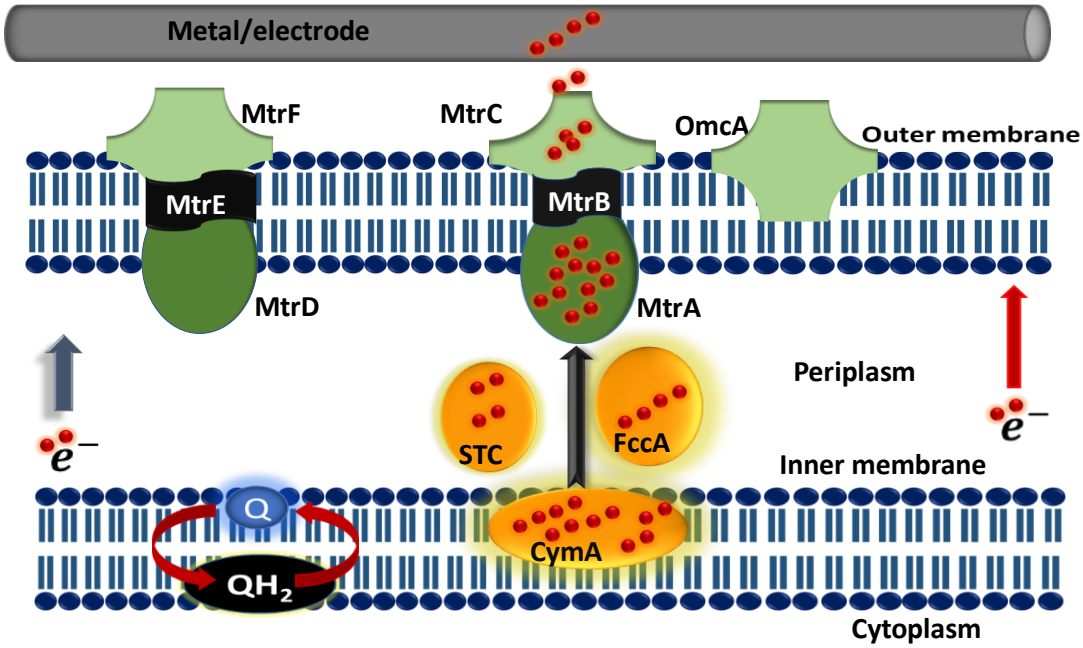

Figure 2. 4 Metal reducing pathways in *S. oneidensis MR-1*

The electron transport channel outside the cell is yet to be understood in most of the microorganisms. However, in *S. oneidensis* MR-1, the metal reduction pathway for the EET has been well defined [145].

Table 2. **2** summarizes the role of multiheme c-Cyts proteins in the metal-reducing pathway.

Table 2. 2 Role of multiheme c-Cyts proteins in the metal-reducing pathway

Multiheme C-Cyts Proteins	Function	Reference
CymA	Oxidation of quinoles in the cell membrane's interior and transfer the free electrons to Fcc3 and STC (in periplasmic space).	[143]
Fcc3 (FccA)	Carrying periplasmic electrons from CymA and passing it to MtrA.	[141]
Small tetraheme cytochromes (STC)	Carrying periplasmic electrons from CymA and passing it to MtrA.	[141]
MtrA, MtrB, MtrD, MtrE	Terminal metal reductases	[146]
MtrC, MtrF	Involved in shifting of electrons from terminal metal reductase to the mineral (Fe III).	[146]
OmcA		
Flavins	Function as diffusive electron shuttle and passes electron from MtrC/OmcA to the mineral surface.	[147]

Shewanella oneidensis MR-1 is the first identified microbe that utilized Mn (III), Mn (IV), and Fe (III) as an ultimate electron acceptor by multistep hopping [148]. *Shewanella oneidensis* MR-1 has a very efficient electron transport system mediated by chemically reduced flavins [147]. The *bfe* gene regulates the release of extracellular flavins and its deletion results in impaired ferrihydrite (Fe (OH)3) reduction in *S. oneidensis* MR-1 [149] . Under anaerobic conditions, flavins bind to the c-Cyt such as MtrC and OmcA and exist in the form of semiquinone. These semiquinones cause an increment in redox potentials, resulting in an

enhanced rate of electron transfer [150]. It has been reported that the diminished reduction rate of Fe (III) exists when flavins are only involved [151].

2.6.1.2 Porin cytochrome pathway in *Geobacter sulfurreducens*

The porin cytochrome pathway has been well understood in *Geobacter sulfurreducens,* which involves MHC proteins. These porin cytochromes play a noteworthy role in the EET. *Geobacter* species reduce Fe (III) and Mn (IV) into Fe (II) and Mn (II). These metals act as terminal electron acceptors [152]. Porin cytochrome complexes (Pcc) are present in *Geobacter sulfurreducens* PCA, which are made up of two MHCs, OmaB/ OmcB and OmaC /OmcC. These two MHCs are attached with a beta-barrel protein named OmbB and OmbC, respectively (Figure 2. 5).

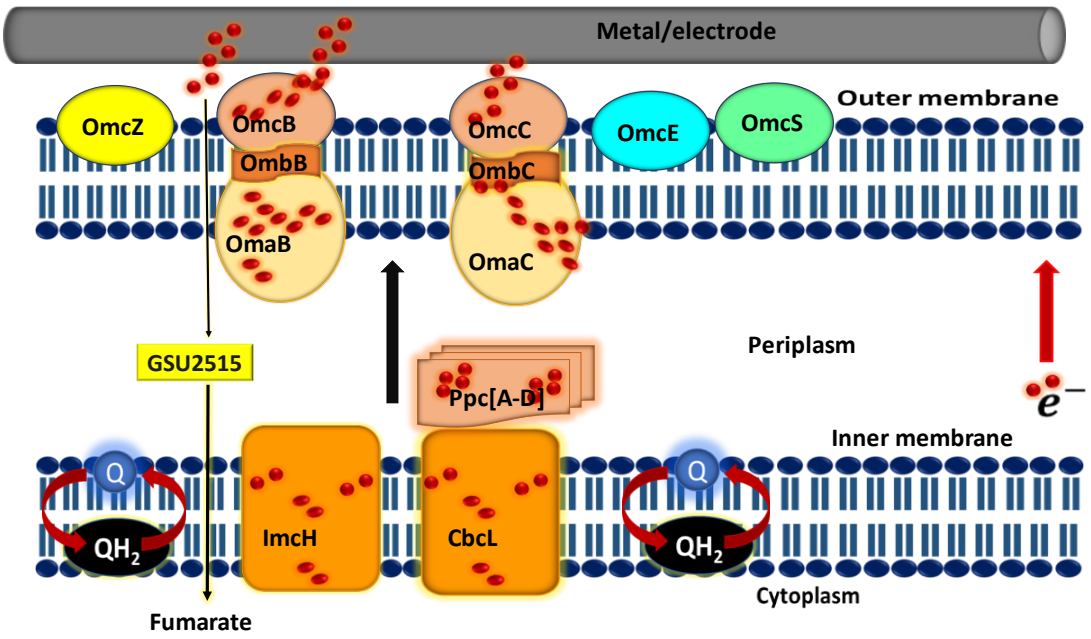

Figure 2. 5 Porin cytochrome pathway in *G. sulfurreducens*

All these cytochromes are implanted in the exterior cell membrane and assist the EET across the outer cell membrane [153]. In the inner cell membrane, the quinone/quinole pool transfer electrons from the inner to the outer cell membrane *via* periplasmic space through nanowires

or c-Cyt redox proteins [154]. In most of the gram-negative bacteria, the outer cell membrane acts as a physical barrier for the electron transport [155]. *G. sulfurreducens* PCA employs Pcc to resolve this barrier. Pcc complex conducts EET across the cell membrane [156]. Pcc consists of:

(1) OmbB/OmbC, a porin similar to outer membrane proteins

(2) OmaB/OmaC, 8-Heme c-Cyt periplasmic proteins and

(3) OmcB/OmcC, 12 heme c-Cyt outer membrane proteins

OmbB/OmbC makes a frame of sufficient length and width across the cell membrane, forming a heme-based channel. OmaB/OmaC and OmcB/OmcC transfer electron across the exterior cell membrane. The knockout gene approach has indicated that OmcS and OmcE are essential for ferric oxide reduction. OmcT is required to stabilize the OmcS [157]. It is often comparable to the metal-reducing pathway (MtrABC complex) model of *S. oneidensis* MR-1 [158]. Pcc proteins are not identical to Mtr complex proteins phylogenetically, although they appear to have similar functions [158]. The OmcS nanowire assists in electron transmission in the biofilm by acting as a conductor but not as a structural component. The OmcZ nanowire contributes to current generation by performing both structural and conductive functions. It has been reported that lowering the pH enhances the conductivity of OmcS and OmcZ nanowires by 100-fold because of protein conformational changes to a β sheet- rich structure. This structural change improves the stacking of hemes in nanowires. Enhanced π stacking between hemes can increase the effective conjugation length, yielding a longer mean free path for electrons that enhances nanowire conductivity [129]. Ye et al. (2022) also reported that the OmcZ nanowire plays both structural and conductive functions, i.e. it contributes in biofilm formation along with current generation. Conductive pili are thought to be involved in long-range electron transfer (LET). Authors reported that that the pili only supports in thick anode biofilm

formation [159]. Table 2. 3 highlights the role of porin cytochromes in cytochrome-mediated pathways.

Table 2. 3 Multiheme c-Cyt proteins involved in porin cytochrome pathway

Multiheme C-Cysts proteins	**Description**	**Reference**
Imc H CbcL	These are putative quinole oxidases present in the cytoplasmic membrane	[152]
Ppc (A-D)	It transfers electrons across the periplasm	[152]
OmaB, OmaC, OmbB, OmbC, OmcB and OmcC	These are trans-outer membrane c-Cyt protein complex; all these cytochromes play a role by carrying electrons from the periplasmic c-Cyts to the superficial surface of the bacterial cell, i.e., terminal metal reductases	[152], [153]

Genomic and proteomic studies carried out in current-consuming *versus* current-producing *G. sulfurreducens* biofilms revealed that there is specific electron transfer component for each direction [160]. Teixeira et al. (2022) observed a novel periplasmic low-spin monoheme cytochrome GSU2515, which was essential for *G. sulfurreducens* electron-harvesting processes. It has been observed that PccH and GSU2515 (periplasmic cytochromes) are overexpressed in *G. sulfurreducens* biofilms**.** Along with PccH, GSU2515 is engaged in the subsequent electron transport activities in the cell. Initially the electrons are taken up by GSU2515 from the outer membrane electron donor, and then undergoes in a reduced configuration. The putative electrons are supposedly transported to PccH. After the delivery of

the electron to PccH, GSU2515 transforms into an oxidized state. These electrons are transferred to a redox partner in the inner membrane by the cytochrome PccH [160].

2.6.1.3 The Pio pathway in *Rhodopseudomonas palustris* TIE-1

Photoferrotrophy is a microbial photoautotrophic metabolism that can fix inorganic carbon into organic one by utilizing photonic energy and reduced Fe (II) as an electron donor for the extracellular interchange of electron [161]. *Rhodopseudomonas palustris* TIE-1 (a purple bacterium) was the first isolated genetically tractable photoferrotroph [161], [162]. Figure 2. 6 shows the phototropic iron oxidation model of electron exchange in *R. palustris* TIE-1.

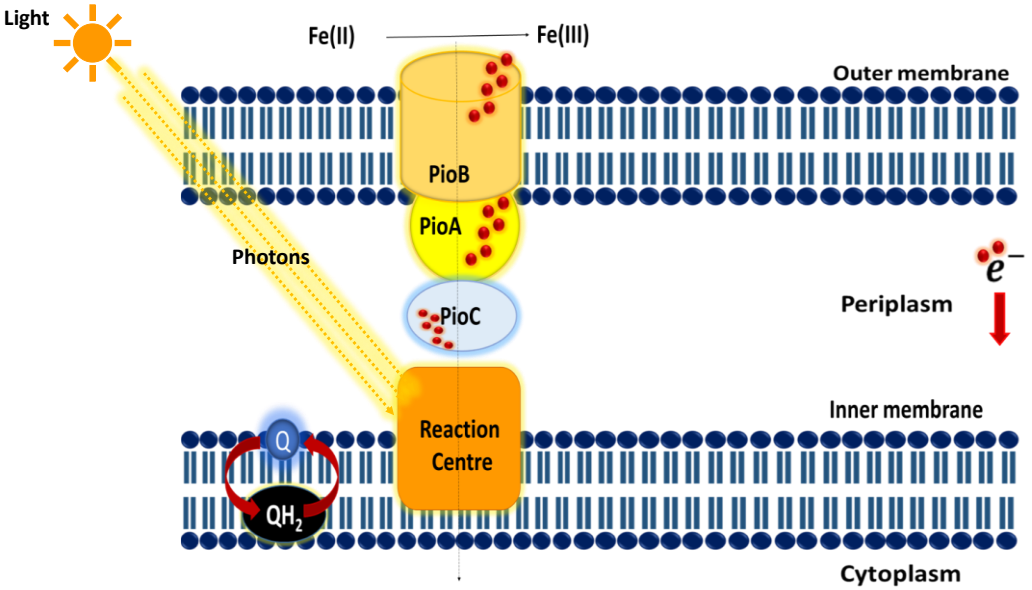

Figure 2. 6 phototropic iron oxidation model in *R. palustris* TIE-1

R. palustris TIE-1 is a phototrophic Fe (II)- oxidizing bacteria that fixes atmospheric CO_2 by utilizing Fe(II) and light energy [163]. Genome *of R. palustris* TIE-1 has a cluster of *pio* genes. It has been reported that the *pio* operon is vital for photosynthetic oxidation of iron, which comprises of *pio A*, a deca-heme c-Cyt (a homolog of mtrA), *pioB* (exterior cell membrane porin and is also mtrB homolog) and *pioC*, which encodes for high redox potential iron-sulfur protein (HiPIP)[162]. It has shown that the deletion of the *pio*-gene cluster disables the *R.*

palustris TIE-1's ability to receive electrons from the electrode [163]. *Pio A* and *Pio B* assist in the oxidation of Fe (II) outside of the cell and allow the released electron to pass through *Pio C* towards the cell interior (periplasm) (Figure 2. 6). *Pio C* relays the electrons from the periplasm to the inner cytoplasmic membrane at the photoreaction center [163], [164]. The relay of electrons through *Pio C* is a light-dependent reaction [164]. High potential redox-active Fe-S proteins (HiPIPs) and c- Cyts are the electron donors for this reaction centers [163].

2.6.1.4 Metal-oxidizing pathway in *Sideroxydans lithotrophicus* ES-1

Acidophilic bacteria, including *Leptospirillum ferrooxidans* and *Acidothiobacillus ferrooxidans* (chemolithoautotrophic families) [165], marine bacteria such as *Gallionella ferruginea,* and freshwater microbes such as *Gallionella capsiferriformans* ES-2 and *Sideroxydans lithotrophicus* ES-1 [166], [167] live at the iron interface. These microbes oxidize iron Fe (II) to reduce oxygen [168]. Figure 2. 7 represents the metal-oxidizing pathway in *S. lithotrophicus* ES-1.

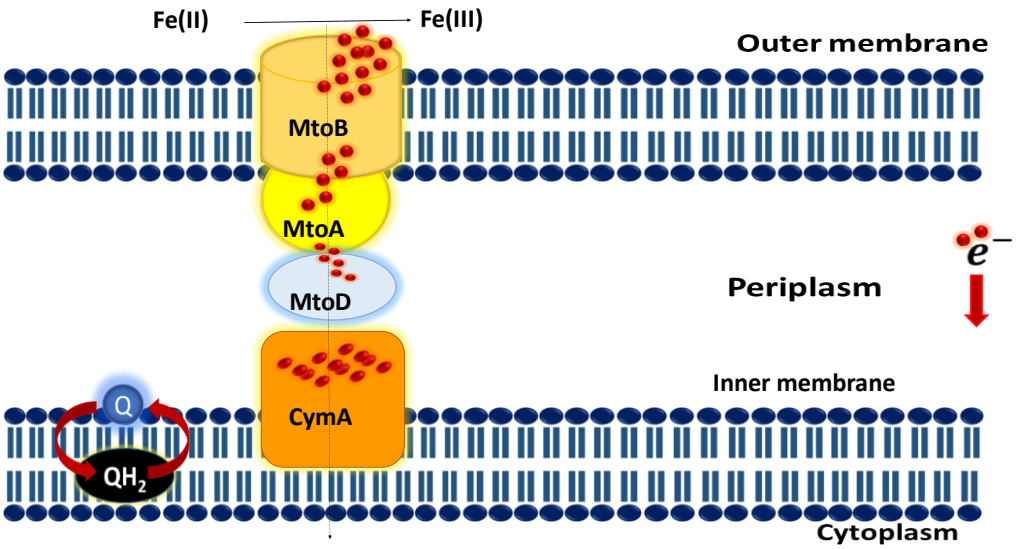

Figure 2. 7. Metal oxidizing pathway in *S. lithotrophicus* ES-1

The metal-oxidizing pathway of neutrophilic *S. lithotrophicus* ES-1 involves the following genes: i- mtoA, which is a Mtr homolog, ii- mtoB, which is MtrB homolog, and iii- mtoD is used for encoding a periplasmic monoheme c type cytochrome [169]. It has been testified that MtoA and MtoB play the same role as MtrA and MtrB and form an electron shuttle that permits the collection of electrons from the Fe (II) oxidation at the cell's exterior facet, which is followed by the transportation of these electrons to the periplasm [170]. MtoA and MtoB involve the direct oxidation of ferrous ions and MtoD is a periplasmic c-Cyt that assists the transmission of the electrons from the MtoA (exterior membrane) to the inner cytoplasmic membrane at CymA (a tetraheme quinole oxidoreductase) [170], [171].

2.6.1.5 Electron transfer pathway in *Thermincola potens JR*

Fe (III) reducing gram-positive *Thermincola potens* JR has several proteins on its cell surface, including multiheme c-Cyts. The enzymatic degradation of these surface-exposed multiheme c-Cyts and other proteins diminish its ferric ion Fe (III) reduction capability [172]. Figure 2. 8 represents the electron transfer model in *T. potens* JR.

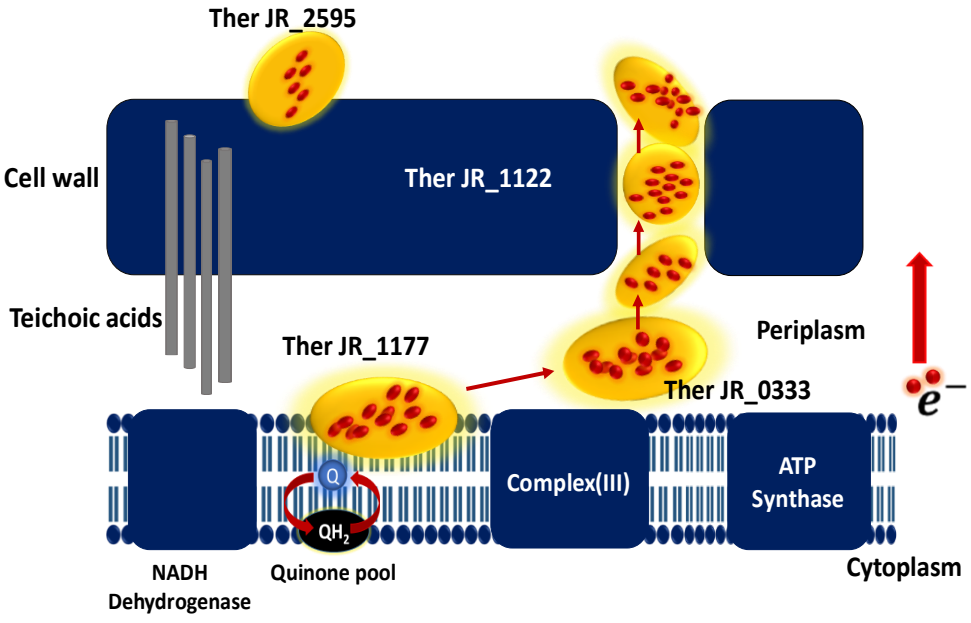

Figure 2. 8. Electron transfer pathway in *Thermincola potens* JR

The electron transfer pathway in *T. potens* JR emphasizes that at least four proteins are involved (TherJR_2595, TherJR_1122, TherJR_1117, and TherJR_0333) in the electron transmission throughout the cell envelope and in the reduction of Fe (III) [173]. TherJR_0333 is a decaheme multiheme c Cyts in periplasmic space. TherJR_1117 is the first MHC of this extracellular cytochrome cascade, found in the inner membrane and acts as quinol dehydrogenase. TherJR_1122 is a hexaheme MHC which is not having any homology to any other characterized MHCs but abundantly present in the electron transfer pathway in *T. potens* JR. TherJR_2595 is a nonheme MHC which is similar to MtrC in *S. oneidensis* MR-I and OmcS in *Geobacter*. TherJR_2595 is exposed to the cell surface of *T. potens* and acts as a terminal metal reductase [173].

2.6.2 EET pathways in Gram-positive bacteria

Recent findings have demonstrated that gram-positive bacteria also play an important role in BES, being able to prevail in electricity-generating communities and under extreme conditions. EET in Gram-positive electrogens can occur either by direct or mediate electron transfer [174].

2.6.2.1 EET pathway in *Lysinibacillus varians* GY32

Lysinibacillus varians GY32 is capable of bidirectional EET. Figure 2. 9 describes EET pathway *in L. varians* GY32. In the inner membrane and periplasm, two c-type cytochrome genes, *T479_RS06590* and *T479_RS20980*, were discovered. *T479_RS20980* diffuses towards the anode surface or donates electrons to redox mediators. For anode respiration, strain GY32 predominantly depends on the release of electron mediators and it secretes and uses cysteine as an electron mediator [175]. Thiol-disulfide oxidoreductase (YkuV) indicates another pathway for the cysteine-mediated EET. Cysteine is a thiol molecule, which is able to reduce iron and manganese oxides in sediment. A key tactic used by bacteria to sense and respond to redox conditions in the environment is the biological production of cysteine and flavins.

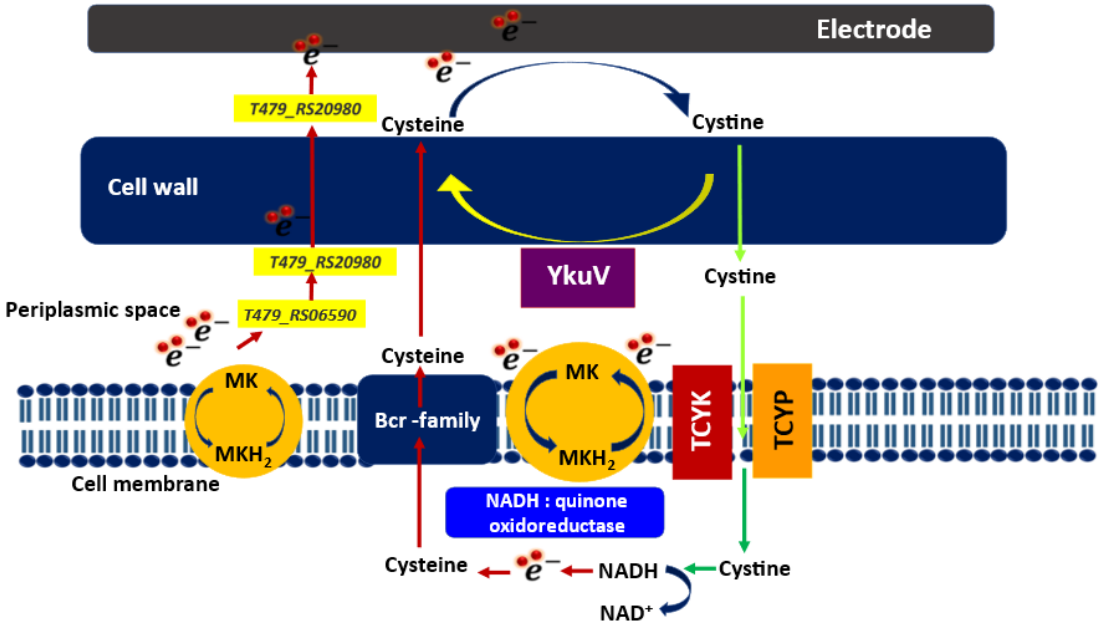

Figure 2. 9 Extracellular electron pathway in *Lysinibacillus varians* GY32

Moreover, *L. varians* GY32 exploits the redox pair cysteine/cystine as an electron mediator. Cysteine has been reduced by some coenzymes such as NADH and menaquinones (MK). YkuV is a periplasmic protein which catalyses the redox reaction of the cysteine/cysteine couple. Cysteine can be transported across the membrane to the outside via cysteine transferase (a Bcr-family protein) and the extracellular cystine can be transferred back to the cytoplasm by symporter TcyP and ATP binding cassette transporters TcyK [175].

2.6.2.2 EET pathway in *Carboxydothermus ferrireducens*

Carboxydothermus ferrireducens is thermophilic anaerobic microbe isolated from a hot spring. *C. ferrireducens* joints the oxidation of organic and inorganic substrate (electron donors) with the reduction of various electron acceptors such as Fe (III), U(VI) and bicarbonate [176]. *C. ferrireducens* does not produce any external soluble electron shuttle to serve as the redox mediators of EET. Multihemes were expected to be localized on the cell membrane or secreted as proteins (Table 2. 4).

Table 2. 4. Multihemes involved in EET mechanism of *C. ferrireducens*

Multihemes	Homology to previously reported cytochromes
Ga0395992_02_112574_114619	Designated as OmhA (an outer multiheme) and homologous to MtrA, OmcB
Ga0395992_02_137389_139104	Designated as SmhA (a Secreted MultiHeme) and Lack homologs among reported determinants of EET
Ga0395992_03_307983_310529	Designated as SmhB (Secreted MultiHeme) and homologous to TherJR_1122
Ga0395992_02_433869_435545	Designated as SmhC and a Secreted MultiHeme
Ga0395992_01_182949_18443	Homologous to OmcC
Ga0395992_01_217646_219709	Gene set for type-IV pili assembly
Ga0395992_01_220048_222057	Gene set for type-IV pili assembly
Ga0395992_01_222075_224618	Gene set for type-IV pili assembly
Ga0395992_02_30996_31874	Homologous to MtrA/MtrD
Ga0395992_02_151260_153788	Homologous to MtrA/MtrD
Ga0395992_01_647313_648317	Homologous to CymA quinol-oxidizing cytochrome
Ga0395992_02_29558_30178	Homologous to soluble secreted cytochromes TherJR_1117, TherJR_0333
Ga0395992_02_135863_137242	Serve as quinol-oxidizing units
Ga0395992_01_684739_686151	Serve as quinol-oxidizing units
Ga0395992_01_187284_188528	Similar to ActA subunit of the respiratory alternative complex III

C. ferrireducens have three multihemes that were encoded in a large cluster consisting of a whole gene set for type-IV pili. This pilin-cytochrome cluster had structural pilin PilA and had have no homology with conductive pilins. No genes were found as relative of Mtr or Pcc type porin cytochrome complex. In *C. ferrireducens* four major c type cytochromes OmhA, SmhA, SmhB, and SmhC are present. OmhA from *C. ferrireducens* have significant sequence similarity with putative terminal reductase OcwA of *T. potens* [176]. OmhA protein found in the cell-metal interface points towards weak bonding of the cytochrome with the cell membrane. The OmhA can either function as a direct electron channel, connecting the cells to the extracellular solid electron acceptor or as electron shuttle near to the cell membrane. The SmhA (octaheme) is related to octaheme tetrathionate reductases (Otr) in Gram-negative bacteria [176].

2.6.2.3 EET pathway in *Enterococcus faecalis*

Enterococcus faecalis is a gram-positive lactic acid microbe, which is able to generate electrons during fermentation and transfer electrons to the anode [177]. If heme is available, then EET route of E. *faecalis* includes the cytochrome bd (quinol oxidase), PplA (flavoprotein), demethylmenaquinone (DMK) pool, and NADH: quinone oxidoreductase. In cytoplasm, electrons generated during glycolysis are transferred towards the cell membrane by membrane-attached dehydrogenases, such as NADH dehydrogenase, that reduce demethylmenaquinone (DMK) [177]. DMK transfers electrons to the cytochrome bd (subunit cydA and cydB). DMK and cytochrome *bd* is essential for efficient EET. Ferric reductase activity is also dependent on DMK. E. *faecalis* cannot produce heme but encodes two heme proteins, catalase and cytochrome *bd*. Enhanced activity of cytochrome *bd* oxidase decreases the reduction of DMK pool within cytoplasmic membrane. In a study, DMK-deficient strain WY84 was grown in the absence of heme (NADH oxidase and cytochrome *bd* activity). DMK-deficient strain WY84 involved NADH dehydrogenase (Ndh3) with the flavin adenine dinucleotide (FAD) on the

cytoplasmic (inner) side of the membrane. DmkAB was found to be involved in the synthesis of a specific quinone. Ndh3 paired NADH (cytoplasm) oxidation with the quinone reduction. Role of Ndh3 was linked with Fe (III) ion reduction by involving EetA and EetB. EetA (membrane protein anchored at the outer side of cytoplasmic membrane) and EetB (integral membrane protein) were found essential for Fe (III) reductase activity. In the presence of active cytochrome *bd* oxidase quinone pool was reduced and this weakened both EET and ferric reductase activity [177].

2.6.3 EET pathway in Yeast

In *Saccharomyces cerevisiae*, both mediator-mediated and direct electron transport occur. *S. cerevisiae* cannot produce redox mediators. Artificial redox molecules are required for EET in yeast. Electrons generated (NAD^+/NADH) during glycolysis are immediately transmitted to the anode via trans-membrane proteins present in the outer cell membrane (Figure 2. 10) [178]

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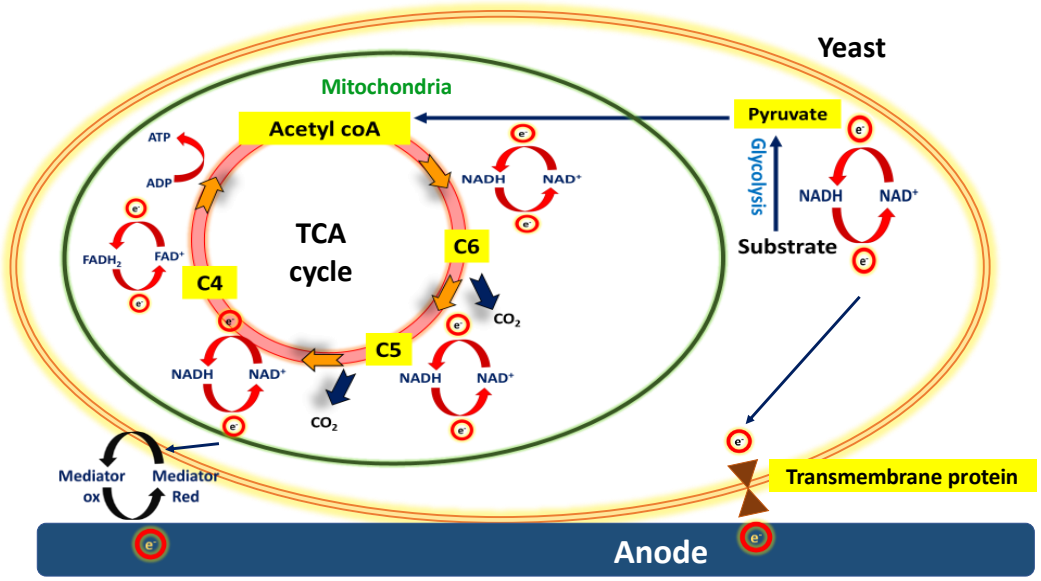

Figure 2. 10. Extracellular electron transfer in yeast

Oxidized mediators get involved in this mechanism and interact with the redox sites such as NADH. This causes the release of the electrons to reduce the mediator (conversion of NADH into NAD^+). Reduced mediators carry these electrons and transfer them to the anode surface, which generates to the flow of extracellular electron from the yeast cells [16]. *Candida melibiosica* is also an electrogenic yeast. *C. melibiosica* is capable to show electrochemical activity in the absence of the artificial mediator (methylene blue, methyl red, riboflavin etc). *C. melibiosica* transfers electrons to the anode via excreted metabolites [16]. *Hansenula anomala* can transport electrons straight to the anode surfaces via redox enzymes, such as lactate dehydrogenase (cytochrome b2), NADH-ferricyanide reductase, NADPH-ferricyanide reductase and cytochrome b5 present in the outer membrane [179].

2.7 Application of EET

2.7.1 Bioremediation of pollutants

Microorganisms such as *Shewanella alga* [180], *Shewanella putrefaciens* [181], *Thauera selenatis* [182], *Shewanella oneidensis*[183] can mitigate water-miscible impurities such as Cr (VI), Tc (VII), Se (IV/VI), and U(VI) by reducing it into Cr (III), Tc (IV), Se (0) and U (IV) [180]–[184]. Table 2. 5 shows list of microorganisms applicable in bioremediation of toxic heavy metals.

Table 2. 5 Bioremediation of toxic heavy metals

Toxic metal remediation mechanism	Microorganism	Reference
Reduction of Cr (VI) to Cr (III)	*Shewanella oneidensis* MR-1	[185]
	Pannonibacter phragmitetus BB	[186]

	Geobacter sulfurreducens PCA	[187]
Soluble U(VI) to insoluble U(III)	*Shewanella oneidensis* MR-1	[183]
	Citrobacter sp.	[188]
	Shewanella RCRI7	[189]
	Shewanella putrefaciens	[190]
Arsenic- Abatement of absorption capacities of arsenic	*Shewanella sp. ANA-3*	[190]–[192]
Se(IV) reduction to Se(0)	*T. selenatis*	[182]
	B. selenitireducens	[193]
V(V) reduced into blue-colored V(IV) and further reduced into V(III)	*P. vanadiumreductans*	[194]
	P. isachenkovii	[195]
Reduction of Mo (VI) into Mo(V).	*Pseudomonas guillermondii*	[196]
	Micrococcus species	[197]
	Enterobacter species	[197]
Reduction of Hg (II) on Fe^{++} dependent mechanism	*T. ferrooxidans*	[198]
	G. metallireducens	[199]

Bacteria such as *Shewanella* and *Geobacter sp*. are helpful in reducing Fe (III), Pd (II), and Se (IV/VI) at the contaminated sites [200]–[202]. *S. oneidensis* MR-1 and *G. metallireducens* GS-15 have been used for bioremediation of U (VI). These bacterial strains can respire in water-miscible U (VI) and reduce it into insoluble U (IV) [203]. *Geobacter sulfurreducens* PCA, not only reduces Fe (III) into Fe (II) in ferrites (solid phase) but also removes (bio-accumulates) Cr (VI) and Tc (VII) from the aqueous phase [204]–[207]. *Geobacter metallireducens* GS-15 oxidizes aromatic compounds such as benzoate, p-cresol, phenol and toluene coupled with simultaneous reduction of Fe (III) [208], [209]. Apart from heavy metals and aromatic compounds metal reducing bacteria also employed for the removal of azo dyes. Azo dyes are derived from benzidine and are carcinogenic. Azo dyes like acid orange (AO-7) and methyl orange have been effectively degraded by electrogens [210], [211].

2.7.2 Biomining

Biomining term is used to define the applicability of microbes to deal with metal-consisting ores by bioleaching and biooxidation. Bioleaching is usually applicable for the base metal extraction, where desired metal is solubilised by microbial action and then recovered from solution. Microbes oxidize the gold and silver containing mineral-sulfide matrix by biooxidation process. When undesirable sulfides are solubilised from the mineral, gold and silver is leached by chemical lixiviants. Table 2. 6 represents recovery of useful metals by microorganisms.

Table 2. 6 Recovery of useful metals

Mechanism	Microorganism	Metal	Reference
	P. islandicum	Gold	[212]
	P. furiosus	Gold	[212]

Reduction of Au(III) (as $AuCl_3$) into insoluble Au(0)	*T. Maritima*	Gold	[212]
	Shewanella alga	Gold	[212]
	G. ferrireducens	Gold	[212]
	Burkholderia contaminant	Gold	[213]
Reduction of $PtCl_6^{2-}$ ions into insoluble platinum	*Shewanella algae*	Platinum	[214]
Reduction of Pd(II) into insoluble Pd(0)	*D. desulfuricans*	Palladium	[215]

At the industrial scale, ferrous ion oxidizing microbes have been used for extracting Cu, Au, Ni, and Zn from the low-grade deposits [216]. *Acidithiobacillus ferrooxidans* oxidizes Fe (II) into Fe (III) and dissolves in Cu (I) by oxidizing it into Cu (II) in copper extraction [217], [218]. Rawlings et al., 2003 recovered soluble Cu (II) from the solution phase [180]. In order to extract gold *A. ferrooxidans* decomposed arsenopyrite ores (gold-bearing) by oxidizing reduced-sulfur compounds [216], [219].

2.7.3 Nanomaterial synthesis

Biogenic nanomaterial generation is advantageous over chemical methods, as it is more capable to produce novel nanomaterials. Nanomaterial biosynthesis needs microorganisms that have ability to reduce broad range of metals or semiconductors. The first phase of nanoparticle biosynthesis is bringing reactant metal into the suitable oxidation state [220]. *Fusarium oxysporum* changes sulfate into sulfide using extracellular sulfate reductase and forms CdS nanoparticle [220]. *Shewanella algae* synthesize periplasmic gold nanoparticle by reducing

gold [221]. *S. oneidensis* initiates nanoparticle formation by its reductase activity, capable to reduce sulfate, palladium and copper[222]–[224]. *Shewanella* species were reported to form arsenic sulfide nanoparticles by arsenic reduction [225], [226]. Similarly, the formation of palladium and magnetite nanoparticle by *Shewanella oneidensis* and *Geobacter sulfurreducens* respectively [200], [227] have been reported. Such nanomaterials are considered biogenic and have broad applications in bioremediation, cancer treatment, semiconductor manufacturing, chemical catalysis, etc. [220].

2.7.4 Bioenergy and biogas generation

Methane can be used as fuel for automobiles, water heaters, ovens, kilns, and turbines, etc. Liquid methane can be used as fuel for rocket when combined with liquid oxygen [228]. Also, it is a main constituent of natural gas and hence useful for electricity generation by using it as a fuel in a steam generator or gas turbine [229]. It has been found that some methanogens have ability to transfer electrons extracellularly. For example, *Methanosarcina barkeri* can accept electrons from the other co-cultivated microbial population [230]. *Methanosarcina horonobensis* and *Methanothrix harundinacea* recover electrons from *G. metallireducens* by interspecies electron transfer [230]. A *Methanobacterium* YSL co-cultured with *G. metallireducens* has been capable of growing by interspecies electron transfer [231]. In *Methanosarcina acetivorans,* the respiration relies on Fe (III) reduction by EET and it is able to oxidize methane anaerobically. Limited concentration of hydrogen or formate induces electro-methanogenesis ability in the *Methanosarcina mazei*. Membrane-bound MHC was recently found in *M. horonobensis* [232] whereas conductive nanowires/pili in *Methanospirillum hungatei* [233]. Another energy source is MFC which is completely based on EET of microorganisms. BES is based on metabolic redox reactions of microorganisms. At anode, microbes oxidize the substrate to generate electrons, protons and CO_2. The protons get transferred to the cathode chamber via proton exchange membrane [234]. The electrons are

directed from anode to cathode via an external circuit for power generation. The electrons and protons are combined to form oxygen and water at cathode. Microorganisms such as *Geobacter sp.* and *S. oneidensis* MR-1 are involved in electron exchange at anode. They are widely used in MFC [234]. In a study, modified anode and mediator increase EET in *S. oneidensis* MR-1 which resulted in approximately ~7-folds increment in current density (1260 mA m^{-2}) [235].

2.8 Yeast as anode biocatalyst

Yeast is a eukaryote having characteristics of an ideal biocatalyst for MFC. Most yeast strains are non-pathogenic and grow on a vast range of organic substrates [236], [237]. The utilization of yeast cells in the anode chamber is mediated by various natural mediators (redox molecules) like azurin, cytochromes, and ferredoxin. These mediators interact with transmembrane proteins or cytochromes for extracellular electron transfer between the yeast and anode [238], [239]. The yeast cell wall comprises of polysaccharides and proteins (100–200 nm) and has a protein-rich cell membrane [240], [241]. In yeast cells, transmembrane proteins and cytochromes are present in the cell membrane and inside the mitochondrial membrane, respectively. Hence, to extract an electron (e^-) out of the yeast cell, a mediator should cross the cell wall and interact with the internal redox molecules such as nicotinamide adenine dinucleotide ($NADH/NAD^+$) and flavin adenine dinucleotide ($FAD^+/FADH_2$) present in the inner mitochondrial membrane [242]–[244]. Figure 2. 11 shows electron transfer during the substrate metabolism inside the yeast cells. Electrons produced during the oxidation ($NAD^+/NADH$) of glucose to pyruvate (glycolysis) are directly transferred to the anode via transmembrane proteins present in the outer cell membrane [245]. Pyruvate is further oxidized into organic acids in the tricarboxylic acid cycle (TCA cycle). The e^- generated during the TCA cycle is accepted by NAD^+ (reduction of NAD^+ into NADH). Oxidized mediators get involved in this mechanism to interact with the redox sites (NADH) and cause the release of the electrons to reduce the mediator (by oxidizing NADH into

NAD^+ again). Reduced mediators lose these electrons at the anode surface, which leads to extracellular electron flow in the yeast cells [239], [245] Figure 2. **12** illustrates mechanisms of electron transfer from yeast cell to the anode.

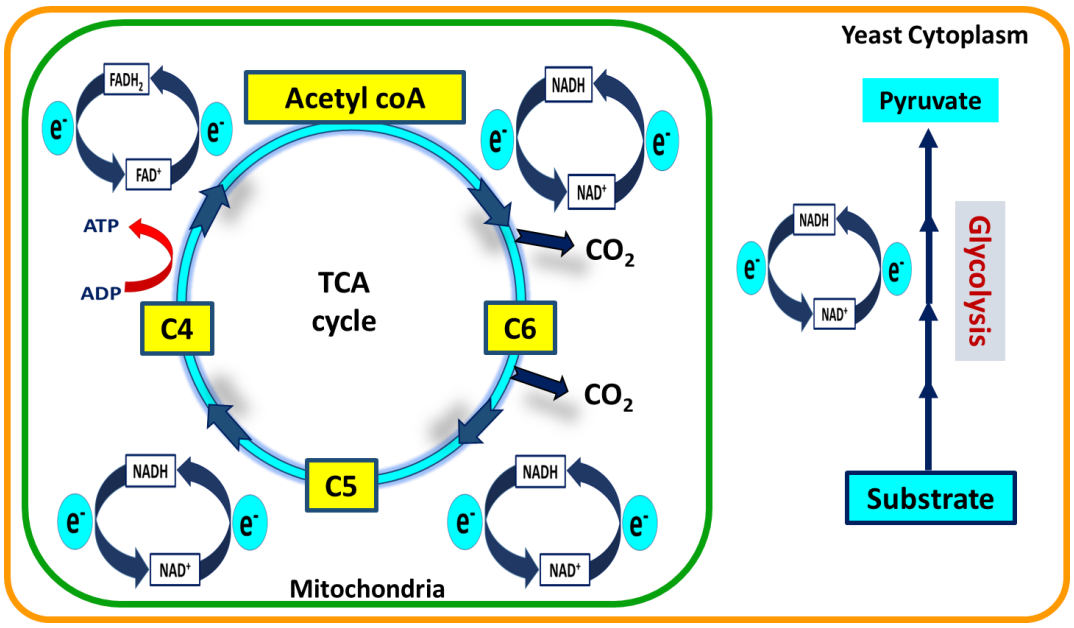

Figure 2. 11. Electron transfer system in a yeast cell

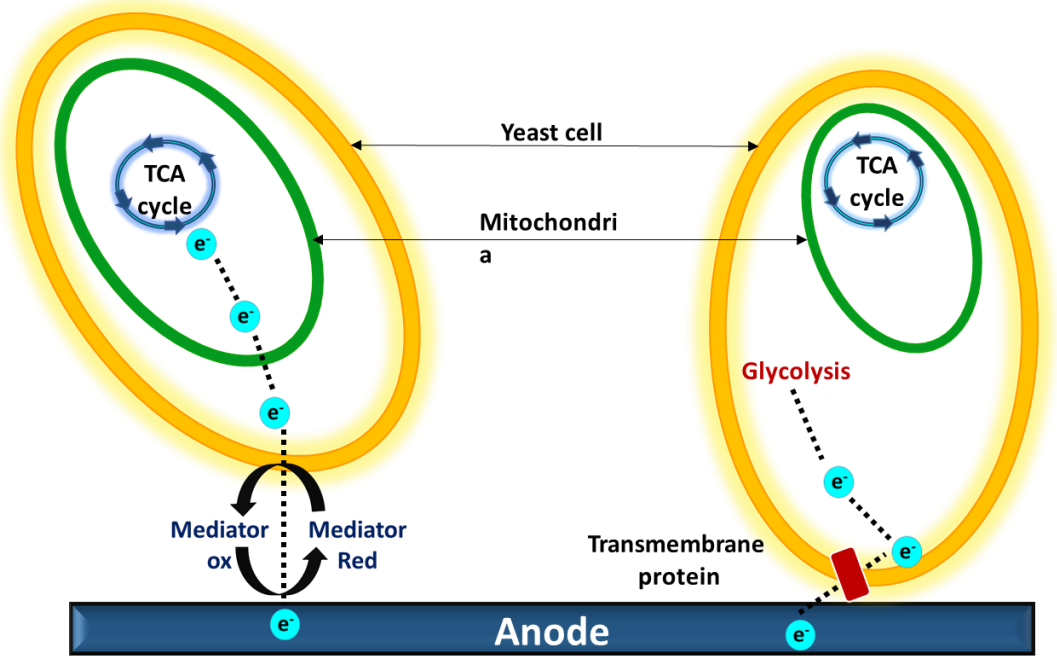

Figure 2. 12. Electron transfer mechanism from yeast cell to the anode.

Several strains of yeast such as *Candida melibiosica* [246]–[248], *Arxula adeninivorans* [249], *Hansenula polymorpha* [250], *Hansenula anomala* [251], *Kluyveromyces marxianus* [252], *Saccharomyces cerevisiae* [237], [242], [253] have been investigated for their electrogenicity. Among these microorganisms, *S. Cerevisiae* has been most widely considered (as the anode biocatalyst) in fungi-based MFCs [236]. One of the serious concerns is to develop new electroactive yeast strains (in terms of genetic engineering) which can compete with bacteria-based MFCs. Usage of yeast cells in MFCs is more pedestrian when complex organic substrates are used as input [236]. The high biodegradation capacity of yeast cells is a critical feature for power generation with concurrent organic waste treatment [236] The major obstacle that needs to be addressed is overcoming lower power generation in yeast-based MFCs as compared to bacterial ones [241], [242]. Yeast has appeared as a decent option for MFC utilization as they are considered as model eukaryotic microorganisms in the laboratory and are broadly applicable at the industrial level for more than a century. Yeast-based MFCs could be a reasonable alternative for treating the rich organic matter in food industry wastewater. The yeast cells present in the effluent of the sugar processing unit, brewery, dairy industry and leather manufacturing unit could be reused in MFC instead of additional microbial inoculum [254] Most of the yeasts are non-pathogenic, have fast growth and high catabolic rates, robust and are easy to handle [249] Yeasts are capable of growing in aerobic conditions over a wide range of substrates, such as glucose, trehalose, maltose etc. Xylose is an abundant saccharide derived from most agricultural residues. *C. slooffiae* JSUX1, a novel yeast strain, is able to use xylose as feed-in MFC and it produces electricity [255] Yeast is tolerant to a broad range of external conditions, the maximum temperature for the growth of most yeasts ranges up to 37 °C [256]. However, results show a noteworthy maximum power density of 30 mW m^{-2} at 55 °C [257]. Researchers isolated temperature tolerant strains of yeast that can even grow at 44 °C and are able to produce ethanol

[258]. Though the anaerobic condition is required at the anode chamber of MFC, yet yeast can survive in both aerobic and anaerobic conditions. Furthermore, a eukaryotic cellular organization with a well-known genome and metabolism helps in processes responsible for power generation in MFC. However, the extracellular electron transfer mechanism in yeast is still uncertain and needs more exploration [259]. Yeast is frequently assumed to be unrealistic option as an anode biocatalyst due to complications with extracellular electron transfer. Yeast-based MFCs are also applicable in bioethanol production plants for onsite current generation [239]. Overall, yeast-based MFCs achieve better performance as compared to cyanobacteria but it has a lower power output as compared to electrogenic bacteria [249].

Researchers reported a maximum power density of 1500 mW m^{-2} by using *S. cerevisiae* as biocatalyst in methylene blue mediated MFC, which is much lesser than the maximum power density of 6860 mW m^{-2} obtained in a mixed consortia MFC [244], [260] Genetic modifications (such as yeast surface display of dehydrogenase enzymes) in the electron transfer mechanism of yeast cells and stacking multiple yeast MFC units in series or parallel combinations might be a possible approach to enhance power generation. Such strategies can assist power supply for the devices with lesser energy requirements, such as LED, charging capacitors and biosensors. Besides these, *S. cerevisiae* can be utilized to produce sustainable biofuels like isoprenoid, bioethanol, butanol and fatty acids [261]

The primary purpose of any MFC is to obtain a certain power level capable of supplying power to particular devices or instruments. There are numerous studies on bacteria, their utilization and MFC optimization to enhance power generation. *S. oneidensis and G. sulfurreducens* are the two most studied metal-reducing microbes for MFC technology. As compared to bacteria, very few studies are performed over yeast-based MFCs, However, some of these studies show significant performance. Table 2. 7 represents the power output of yeast, electrogenic bacteria and their co-culture in MFC.

Table 2. 7 The power output of some yeast, bacteria, and their co-culture in MFC.

Microbes	MFC Type	Electrode	Mediator	Power density (mW m^{-2})	Reference
A. adeninivorans	Two chamber MFC continuous mode	Carbon fibre cloth	2,3,5,6-tetramethyl-1,4-phenylenediamine	1000	[249]
C. slooffiae strain JSUX1	Dual-chamber MFC	Carbon felt	Riboflavin	67	[255]
C. fukuyamaensis	Dual-chamber MFC	Boron-doped diamond electrode	-	425,82	[262]
K. marxianus	Dual-chamber MFC	Carbon rods	2-hydroxy-1,4-naphtoquinone	22	[252]
Lipomyces starkeyi	Two-chamber MFC	Stainless steel	-	47.6	[263]
S. cerevisiae	Borosilicate glass titration vessel	Graphite rod	9,10-phenantrenequinone	22.2	[264]
S. cerevisiae	MFC with rotating disc electrodes	Carbon felt	Methylene blue	1500	[244]

G. sulfurreducens	Two-chamber MFC	Carbon felt	Fumarate	16. 2	[265]
S. oneidensis MR-1	Single-chamber MFC		Ferric citrate	158.1	[266]
K. pneumoniae	Two-chamber MFC	Stainless steel	-	95.3	[263]
Wild S. cerevisiae with S. oneidensis	H shaped Glucose-fed MFC	Carbon cloth	-	71.5	[267]
Recombinant S. cerevisiae with recombinant S. oneidensis	H shaped Glucose-fed MFC	Carbon cloth	-	123.4	[267]
L. starkeyi with K. pneumonia	Two-chamber MFC	Stainless steel	-	286	[263]

Yeast-based MFCs have lower power output as compared to electrogenic bacteria. However, several studies have reported significant outcome involving yeast with mediators in MFC [244], [262]. A study reported a maximum power density of 1500 mW m^{-2} by using *S. cerevisiae* as biocatalyst in methylene blue mediated MFC [244]. Researchers utilized *C. slooffiae strain JSUX1* for bioelectricity and biohydrogen from xylose fed MFC and obtained a power density of 67 mW m^{-2} [255] Utilizing yeast opens a broad possibility for carbon substrates to be used in MFCs, such as xylose and cellulosic biomass with an additional benefit of bioethanol and biohydrogen production [255] In a study, *Candida fukuyamaensis* used as anode biocatalyst. Dual-chamber MFC was operated at different pH and temperature for 3 h

and the maximum power density of 425,82 mW m^{-2} at pH 7.5 without a mediator was obtained [262]. Researcher, compared *L. starkeyi* (yeast), *K. pneumonia* (bacteria) and *L. starkeyi- K. pneumonia* co-culture for power generation without exogenous mediators. The co-culture of *K. pneumonia* and *L. starkeyi* attained a power density of 286 mW m^{-2}, which was several folds greater than the bacteria and yeast [263]. Yeast can be an efficient biocatalyst in MFC similar to electrogenic microbes. More attention is required towards their optimization, strain improvement and scale-up for efficient application.

MFCs have gained significant attention from researchers as they involve various microorganisms such as bacteria, microalgae and fungi as biocatalysts. They utilize an extensive range of organic substrates (from simple saccharides to highly contaminated toxic wastewater) and provide a sustainable route for future power generation strategies. During the last decade, many researchers have applied *S. cerevisiae* as a biocatalyst at the anode in the MFCs (with or without exogenous mediators) [242], [244] Various mediators such as thionine [268], methylene blue [253], riboflavin [257] and neutral red [269]have been added externally to the anode chamber for enhancing electron transfer from the yeast cell. Researchers explored the electron transfer in *S. cerevisiae*-based MFCs by using a mediator less air cathode [238]. **Table 2. 8** represents yeast cell utilization (without mediators) in MFCs and their performance.

Table 2. 8 Performance of yeast cells (without exogenous mediators) in MFC

Yeast	MFC type	Substrate	Anode	Cathode	Power density mW m^{-2}	Reference
S. cerevisiae	Dual chamber	Lactose	Graphite plate modified with MWCNT	Graphite plate	2.7	[241]

CtCDH-displaying *S. cerevisiae*	Dual-chamber	Lactose	Graphite plate modified with MWCNT	Graphite plate	33	[241]
S. cerevisiae	Air cathode	Glucose	Au-sputtered carbon paper	Pt-carbon paper	2	[242]
S. cerevisiae	Air cathode	Glucose	Co- sputtered carbon paper	Pt-carbon paper	20.2	[242]
S. cerevisiae	Air cathode	Glucose	Carbon paper	Pt-carbon paper	12.9	[242]
S. cerevisiae	Air cathode	Synthetic wastewater	Graphite plate	Graphite plate	25.51	[245]
S. Cerevisiae and mixed culture	Dual chamber	Glucose	Graphite plates	Graphite plates	28	[268]
C. melibiosica	Dual chamber	Fructose in YEPD	Carbon felt	Carbon felt	20	[270]
C. melibiosica	Dual chamber	Fructose in acetate buffer	Carbon felt	Carbon felt	45	[271]
C. melibiosica	Dual chamber	Fructose in YEPD	Carbon felt	Carbon felt	27	[272]
A. ıdeninivorans	Dual chamber	Glucose in YEPD	Carbon fiber cloth	Carbon fiber cloth	70	[249]

Candida melibiosica 2491, having high phytase activity has been used in the absence of artificial mediators with the different substrates (fructose, glucose, sucrose) [246]. It was also observed that the rate of substrate consumption, yeast cell growth phase and current production

are interrelated with each other [246], [270]. *Arxula adeninivorans* (tolerant to high salinity and temperature up to 48 °C) was investigated for their bio-electrochemical properties [249]. *A. adeninivorans* were utilized in a double chamber MFC in the absence of an exogenous mediator. A maximum power density of 28 mW m^{-2} was obtained using *A. adeninivorans* [249] A study investigated the electrogenic activity of yeast strains such as *Candida glabrata, Hansenula polymorpha, Schizosaccharomyces pombe, Pichia pastoris, Kluyveromyces marxianus, Saccharomyces cerevisiae* and *Kluyveromyces lactis* in a dual-chamber MFC in the absence of exogenous mediators [252]. Additionally, *S. cerevisiae* and other yeast strains have been used effectively as an anode biocatalyst without mediators. However, the power output obtained was very low and unstable [238], [241], [242]. Less electron transfer rate from the yeast cell to the anode is the main reason for unstable power generation. Researchers have made progressive efforts to enhance the performance of yeast-based MFC systems. Adding exogenous mediators is the most common approach to enhance extracellular electron transfer in the yeast cells [237], [253], [273]. Apart from this, researchers have also focused on electrode modification [242], MFC designs and genetic modification in yeast cells to achieve the effective and enhanced application of yeast-based MFC systems [240], [241].

2.8.1 Factors affecting the performance of the yeast-based MFCs

Yeast-based MFCs may be applicable for devices with minimum power requirements. Using charging capacitors/batteries is another option to store generated power for a stable direct current supply. It is vital to recognize the crucial factors influencing the performance of MFC. Amid various factors prompting the MFC performance, the architecture of the MFC, types of microbes and their metabolic activity, substrate, extracellular electron transfer, anode/cathode

material, electron acceptor at the cathode, cation exchange membrane, mediators and other operating conditions (pH, temperature) are significant factors. Studies evaluated the performance of a yeast-based MFC with platinum mesh electrodes in batch mode [257]. Authors altered the concentration of the yeast cells, anaerobic and aerobic states, substrate concentration, temperature and agitation rate. Results approved the recognized traits of the yeast cell. Glucose (substrate) concentration up to 0.1 M had a moderate current output. The elevated glucose concentration up to 0.5 M showed the "crabtree effect", which suppressed power output to an extent. Limited diffusion of the redox mediator resulted in an inefficient electron transfer. Agitation in the anode chamber increased the current generation by prompting enhanced diffusion of redox mediators[257]. Generally, under anaerobic conditions, the fermentation pathway delivers fewer electrons per unit of glucose. Still, anaerobic conditions provide better current generation as electrons from the cell directly moved to the anode surface. The electron generation rate per unit of glucose was greater under aerobic conditions [257]. These ideal circumstances might change if the MFC operation remains for a more extended duration. High cell concentration, accumulated by-products and cell death may influence MFC's power output. The maximum temperature for the growth of most yeast cells is up to 37 °C [256]. However, Walker and Walker (2006) reported a noteworthy maximum power density of 30 mW m^{-2} at 55 °C. Sree et al. (2000) isolated temperature tolerant strains that can grow at 44 °C and are able to produce ethanol. However, when the temperature increased up to 44 °C, the ethanol and biomass yield reduced significantly [258].

2.8.2 Strategies to enhance the electron transfer from yeast cell to the anode

Yeast can be used as an effective anode biocatalyst in MFC [236], [274]. Power output in yeast-based MFCs is often limited by lower electron transfer rate and hence current researches on yeast-based MFCs involve those techniques which may enhance the rate of electron transfer to the anode. Following techniques are used for enhancing the efficiency of yeast-based MFCs:

i. Addition of artificial mediators [237], [253], [273].

ii. Anode surface modification [275].

iii. Yeast cell immobilization [240]

iv. Yeast surface display method [241].

v. Genetically modified yeast cell [276].

2.8.2.1 Addition of artificial and natural mediators

Exogenous mediators are primarily utilized to enhance electron transfer to the anode in MFCs mediated by electrogenic bacteria and yeast cells. A suitable exogenous mediator must have the following properties [277], [278]

i. Capable of penetrating the cell membrane

ii. It must be helpful in liberating the electrons quickly towards the anode

iii. Nontoxic and appropriate for yeast cell growth

iv. Should leave the yeast cell easily via the cell membrane

v. Chemically stable and soluble in the anolyte.

Mediators such as bromocresol green, methyl orange, neutral red, thionine, methylene blue, yeast extract and riboflavin are used in various yeast-based MFCs [270], [279], [280]. Table 2. 9 represents the utility of yeast cells with mediators in terms of efficient performance in MFCs.

Table 2. 9 Utilization of yeast cells (with exogenous mediators) in MFC.

Type of MFC	**Yeast**	**Substrate**	**Mediator**	**Anode**	**Cathode**	**Power output (mW m^{-2})**	**Ref**
H-type Dual-chamber	*S. cerevisiae*	YEPD with Glucose	Methylene Blue	Carbon felt modified	Un-treated carbon	429.29 ± 42.75	[280]

				with poly-ethyleneimine	felt		
H-type Dual-chamber	*S. cerevisiae*	YEPD with Glucose	Methylene Red	Carbon felt modified with poly-ethyleneimine	Un-treated carbon felt	282.77 ± 15.95	[280]
Dual-chamber	*P. fermentans*	YEPD broth medium	Methylene blue	Carbon fibers	Stainless steel wire	12.3	[279]
Single-chambered	*P. fermentans*	YEPD broth	Methylene blue	Carbon fibers	Stainless steel wire	16.4	[279]
Dual-chamber	*C. slooffiae strain JSUX1*	Xylose	Riboflavin	Carbon felt	Carbon felt	67	[255]
Dual-chamber	*S. cerevisiae*	Glucose	Methylene Blue	Platinum mesh	Platinum mesh	65	[257]
Dual-chamber	*S. cerevisiae*	Glucose	Methylene Blue	Copper electrode	Copper electrode	4.48	[253]
Dual-chamber	*S. cerevisiae*	Glucose	Thionine	Graphite plate	Graphite plate	60	[268]
Dual-chamber	*S. cerevisiae*	Glucose	Neutral red	Graphite plate	Graphite plate	133	[281]
Dual-chamber	*S. cerevisiae*	Glucose	Riboflavin	Graphite	Graphite	33	[269]

Two-Chamber MFC	*C. melibiosica*	YEPD with Fructose	Bromocresol green	Carbon felt	Carbon felt	46	[270]
Two-Chamber MFC	*C. melibiosica*	YEPD with Fructose	Methyl orange	Carbon felt	Carbon felt	137	[270]
Two-Chamber MFC	*C. melibiosica*	YEPD with Fructose	Methyl red	Carbon felt	Carbon felt	113	[270]
Two-Chamber MFC	*C. melibiosica*	YEPD with Fructose	Neutral red	Carbon felt	Carbon felt	89	[270]
Two-Chamber MFC	*C. melibiosica*	YEPD with Fructose	Methylene blue	Carbon felt	Carbon felt	640	[270]

Table 2. 9 validates the usage of exogenous mediators to enhance power output in yeast-based MFC systems. *Candida melibiosica 2491* possesses electrogenic properties and is utilized as a potential biocatalyst at the anode in the yeast-based fuel cells [270] *Candida melibiosica* 2491 generates a power output of 20 mW m^{-2} in a mediator-less MFC. In order to raise the power generation efficiency of *Candida melibiosica* 2491 based MFC, several exogenous mediators such as bromocresol green, neutral red, methyl orange, methylene blue and methyl red have been used, which yielded the maximum power density as 46 mW m^{-2}, 89 mW m^{-2}, 113 mW m^{-2}, 137 mW m^{-2} and 640 mW m^{-2}, respectively [270]. The effect of methylene blue and methylene red was investigated on *S. cerevisiae*-based yeast fuel cells. Authors combined both indirect and direct electron transfer mechanisms and found that methylene blue (429.29 ± 42.75 mW m^{-2}) was a far better mediator for MFC than methylene red

(282.77 ± 15.95 mW m^{-2}) [270]. The electrogenic property of *Pichia fermentans* in two different MFC setups (single-chambered and dual chamber) with and without a mediator (methylene blue) was investigated [279]. The maximum power density obtained in a dual-chamber setup without a mediator and with methylene blue was 4.07 mW m^{-2} and 12.3 mW m^{-2}, respectively [279]. Maximum power density in a single-chamber setup obtained without a mediator and with methylene blue is 6.43 mW m^{-2} and 16.4 mW m^{-2}, respectively [279]. The major downside of using exogenous redox mediators is that continuous addition of these is needed to achieve higher power generation efficiency in the MFC system. This surges the cost of power production and is non-eco-friendly. These shortcomings can be overcome by immobilizing redox mediators on the surface of the anode. Nevertheless, MFC operation of long duration may lead to detachment of immobilized redox mediators from the anode surface. Hence, it is needful to look for clean alternatives to enhance the efficiency of yeast-based MFC [240]–[242]

2.8.2.2 Yeast extract as a sustainable mediator

Though exogenous mediators are expensive and might be toxic to cells after a specific limit, yet a few yeast strains are electrochemically active without external redox mediators such as *A. adeninivorans* [249], *C. melibiosica 2491* [246], *H. polymorpha* [250], *H. anomala* [251], *K. marxianus* [252] , *S. cerevisiae* [240]. Electrogenic ability in yeast is related to the mediators secreted by the yeast itself. Several natural mediators like ferredoxin, azurin and few cytochromes are present in the yeast extract could be effectively utilized as mediators [237]. Authors used yeast extract as a natural electron mediator in *S. cerevisiae*-based MFC with gold-plated carbon paper and unmodified carbon paper anodes separately. After adding yeast extract, an increase in power density from 12.9 to 32.6 mW m^{-2} for plain carbon paper anode was observed. On the other hand, in the gold-plated anode, power density rose significantly from 2 to 70 mW m^{-2} [237]. Further, the addition of some natural redox

mediators such as flavins, quinones and phenazines could also be a good option for enhanced electron transfer.

2.8.2.3 Anode surface modification

The anode chamber is the most crucial asset in the MFC setup and it is vital for the efficient action of the MFCs. Anode chamber acts as the place where microbes are cultured, while anode works as a surface where biofilm is formed by biocatalyst (microorganism) and captures electrons via extracellular electron transfer from the cell membrane to generate power [59], [282]. The construction material of the anode also influences the cost of the MFC. Ideal anode material must be [59], [282], [283]:

i. Highly conductive

ii. Biocompatible

iii. Affordable

iv. Easily and readily available

v. Should have excellent microbial adhesion properties.

The boron-doped diamond (BDD) anode has been used with a yeast biocatalyst *Candida fukuyamaensis* in an MFC (without mediator at pH 7.5) and maximum power and current density obtained was 425,82 mW m^{-2} and 440 mA m^{-2}, respectively [262] BDD is a material that is commonly used as an electrode in electrochemistry owing to its biocompatibility, good stability and specificity for oxygen [262] The power generated by the BDD anode in MFC was without adding a redox mediator, which might be due to the direct transfer of electrons from the *Candida fukuyamaensis* cell to the anode through physical attachment between the yeast cell membrane and anode. At pH 7, the lowest power generation was observed (in BDD electrodes) as the isoelectric pH of redox protein is in close vicinity, which neutralizes the charge. Power generation relies on extracellular electron transfer mediated by redox protein (within the cell membrane) as it is influenced by pH variation [262] The behaviour of rough-

surfaced gold nanoflower grown on the top of polyethyleneimine functionalized carbon felt (CF-PEI) anode has been also observed [284] Gold nanoparticle (AuNPs) growth was achieved by preparing a solution consisting of L-ascorbic acid (gold salt reducer), 4-mercaptobenzoic acid (strong ligand) and chloroauric acid (nanoparticle initiator). AuNPs decorated CF-PEI (CF-PEI-AuNPs) generated a maximum power density of 2771 mW m^{-2} (with 715 μM 4-mercaptobenzoic acid for 30 min), which is a benchmark in the field of yeast-based MFCs [284]. Quorum sensing is an interesting capability of microbes to regulate their response towards the external surrounding and channelize signalling with nearby cells [285]–[287] In MFCs, biofilm development is a central function for power generation [286], [287] Quorum sensing is considered a natural mechanism for enhancing current generation [285] Phenylethanol, tryptophol and tyrosol have been separately immobilized on the carbon felt surface (anode) to strengthen biofilm attachment to the anode, which expedites direct electron transfer in *S. cerevisiae*-based MFC [288] Electrochemical impedance spectroscopy and optical inspection have shown that biofilm accumulation is homogenous between the carbon felt fibers [288] MFC employing tryptophol–CF–PEI and phenylethanol–CF–PEI showed almost similar maximum power density of 156.57 ± 5.84 mW m^{-2} and 159.46 ± 10.68 mW m^{-2} whereas tyrosol–CF–PEI reflected comparatively lower maximum power density of 135.56 ± 3.79 mW m^{-2}, which depicted that tryptophol and phenylethanol were more effective than tyrosol [288]

The yeast-based MFC mediated by modified CF-PEI as anode consisting of manganese oxide decorated iron oxide nanoflowers (FeMnNPs) was explored by [275] The growth of FeMnNPs via surface-bound iron (Fe) particles was investigated with and without surfactant ligand in an aqueous solution. Rough, uniquely shaped nanocrystal growth was observed inside the CF-PEI fibers (hydrophilic surface). The anodic viability of FeMnNPs–CF–PEI was explored through simulation and maturation of *S. cerevisiae* [275]. The FeMnNPs were grown

in the presence of sodium dodecylbenzene sulfonate (surfactant ligand). The influence of iron and manganese ions was examined to evaluate the yeast viability, biofilm formation and electrochemical properties. The maximum power density obtained from unmodified CF-PEI, FeMnNPs–CF–PEI (without surfactant) and FeMnNPs–CF–PEI (with surfactant mediated growth technique) was 380.0 mW m^{-2}, 3600 mW m^{-2} and 5838 mW m^{-2}, respectively [275]. Similarly, cellulose microcrystalline and microfibrils were immobilized on the surface of the carbon felt by applying carrageenan [289] The maximum power density obtained from cellulose carrageenan modified carbon felt (70.98 mW m^{-2}) was greater than the one obtained by using plain carbon felt (48.38 mW m^{-2}) as an anode. Cellulose donated proton (H^+) and formed oxycellulose (having COO^- group), which resulted in enhanced direct electron transfer between *S. cerevisiae* and the anode. Cellulose carrageenan modified carbon felt anode structure has shown exemplary performance in MFC [255]. The polyurethane sponges impregnated with carbon nanotubes as an anode in a dual-chamber MFC consisting of a proton exchange membrane were investigated [290] This assembly was able to acquire a maximum power density of 100 mW m^{-2} [256]. A low-cost anode, the graphite-cement composite (GCS) was developed, applied and investigated in yeast-based MFC [291]

GCS was prepared by blending and hardening cement-fumed SiO_2 and graphite. The GCS has a pore size ranging from micro to nanopores, has a large surface area and superior electrical conductivity. The GCS anode was attached firmly with yeast biofilm and gained a maximum power density of 329.8 ± 76.1 mW m^{-2} for GCS [291]. Carbon felt is flexible, porous, inexpensive and has conductive three-dimensional support. Unmodified carbon felt has some flaws such as deformation by pore compression, hydrophobicity, lower biofilm adhesion and fragility by getting aged. Polyethyleneimine (PEI) is used to coat the carbon fibers (carbon felt coated with polyethyleneimine) [240], [292]. PEI has the ability to link carbon and biocatalysts together. It has been reported that CF-PEI doped with surfactant FeMnNPs

achieved greater power density (5838 mW m^{-2}) than the CF-PEI doped with surfactant AuNPs (2771 mW m^{-2}) [275]. The high-power output from FeMnNPs–CF–PEI anode was due to development of a highly efficient electrochemical interface complemented with sodium dodecylbenzene sulfonate (anionic surfactant mediator) between the yeast biofilm and FeMnNPs-CF fibers [275] Anode material plays a crucial role and controls the power generation in MFC. The nanocomposite offers enormous chances to use for anode modification purposes [59] Oxide of titanium, tin, iron, and manganese exhibited noteworthy increments in the power output when used in their nanocomposite form. This showed the presence of a synergistic effect [283], [293] Anode modification generally involves introducing functional groups such as quinoid, amide on its surface, which is helpful in establishing interactions between the yeast cell wall and functional groups on the anode surface [293]. It is necessary to achieve functional group stability on the surface of the anode, which is suitable in the long run of MFC operations and assists in high-power production.

2.8.2.4 Genetically modified yeast

Developing genetically engineered yeast cells is a potential approach for improving the performance of the yeast-based MFCs. Researchers developed *S. cerevisiae* for lactate production by inhibiting ethanol generation [267]. Authors used recombinant *S. cerevisiae* with *S. oneidensis* consortium in which lactic acid pathway from bovine was incorporated into *S. cerevisiae* and ethanol pathway was knocked out from *S. cerevisiae* to inhibit ethanol synthesis. Alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) are two main enzymes involved in the ethanol synthesis. Deletion of three PDC genes (PDC1, PDC5, PDC6), two ADH genes (ADH1, ADH4) interrupted the ethanol pathway in *S. cerevisiae* [267]. L-lactate dehydrogenase (LDH) catalysed the pyruvate reduction to synthesize L-lactate, where NADH was oxidized into NAD^+ to balance the intracellular redox in *S. cerevisiae*. The glucose was metabolized by *S. cerevisiae* and it produced lactic acid,

which was used by *S. oneidensis* for the enhanced power generation from MFC[267]. As a genetic approach, yeast surface display has been significantly studied to enhance power output. Yeast modifications such as surface-displayed glucose oxidase (GOx) and enzyme dehydrogenases are much explored [241], [294]

2.8.2.5 Yeast surface display technique

Yeast surface display is a method of representing recombinant proteins by genetic fusion on the surface of the yeast cell wall. Pyranose dehydrogenase (isolated from *Agaricus meleagris*) and cellobiose dehydrogenase (isolated from *Corynascus thermophiles*) were displayed on the surface of *S. cerevisiae.* These surface-displayed dehydrogenases were used with graphite plates altered with multi-walled carbon nanotubes (MWCNT) as anodes to enhance the power efficiency of MFC [241]. Unmodified *S. cerevisiae* gained the power density of 2.7 mW m^{-2}, which was compared with modified yeast. The result indicated that modified *S. cerevisiae* enhanced power output approximately 12 times more than the unmodified one. Surface displayed cellobiose dehydrogenase reflected the power output of 33 mW m^{-2} with lactose as a substrate in a mediator less dual-chamber MFC while surface-displayed pyranose dehydrogenase achieved a power output of 39 mW m^{-2} with D-xylose [241]. The glucoamylase from *A. niger* was displayed on the *S. cerevisiae* [294]. Glucoamylase is an enzyme that digests starch into a carbohydrate monomer (glucose). When glucoamylase was coupled with glucose oxidase (GOx), glucose oxidation took place in the anode chamber [294]. Expression of glucoamylase was achieved through the a-agglutinin yeast surface display system. When a macroalgae *Ulva lactuca* was fed into MFC containing glucoamylase and GOx displayed mixed yeast culture (in anode chamber), it showed a power density of 18.0 mW m^{-2}, which was higher than the glucoamylase expressing yeast culture containing purified glucose oxidase (8.0 mW m^{-2})

[294]. The use of GOx displayed yeast as an anode biocatalyst in MFCs has shown remarkable outcomes as compared to wild *S. cerevisiae*. Authors reported GOx (redox enzyme) on the surface of *S. cerevisiae*. GOx displaying yeast obtained power density of 13.6 mW m^{-2}, which was much higher as compared to the power density of 7 mW m^{-2} obtained from wild yeast [276].

2.8.3 Applications of yeast-based MFCs

Yeast based MFC is an advanced technology that has the potential for energy production and deals with the whole dimensions of the fossil fuel crisis [236], [275], [290], [291]. It is forthcoming expertise in wastewater treatment along with power production, which utilizes organic feed as a substrate for yeast and generates bio-electricity.

2.8.3.1 Electricity generation

Tremendous research work has been reported on the electroactivity and mechanism of extracellular electron transfer in the yeast cells [253], [259], [280] However, most of these studies focus on the addition of mediators or modification in electrode materials to enhance the power generation efficiency of yeast-based MFCs [275], [284], [289], [295] Due to limited direct electron transfer in yeast cells, power output in yeast-based MFCs is low as compared to the MFC catalysed by electrogenic bacteria [29], [267]. Table 2. 8 and Table 2. 9 represent the maximum power density achieved by various yeast strains as an anode biocatalyst (with or without mediators) in various combinations of electrodes and substrates used in MFC. It is evident that S. *cerevisiae* is the most popular strain which has been used in yeast-based MFCs [241], [242], [245], [273] This is due to their broad substrate spectrum, affordability, non-pathogenic character, easy and rapid mass cultivation, can be stored or maintained for a long duration in the form of dried powder together with bioethanol production [241], [242], [274].

2.8.3.2 Alcohol production

It is well known that the oxidation of organic compounds yields ethanol under anaerobic conditions in the yeast cell metabolism. In anaerobic conditions, the glycolysis pathway produces 2 mol of pyruvate from 1 mol of glucose. Enzyme pyruvate decarboxylase further transforms 2 mol of pyruvate into 2 mol of acetaldehyde [239]. These two moles of acetaldehydes are further converted into 2 mol of ethanol by an NADH-dependent enzyme alcohol dehydrogenase. 2ATP (Adenosine triphosphate), $2H^{+,}$ and $2e^{-}$ are obtained in order to reduce NAD^{+} into the NADH. However, the oxidation efficiency of NADH is very slow in the process of fermentation, so an excess of NADH is accumulated inside the yeast cell. Such NADH accumulation halts the metabolic activity of yeast [239]. MFC setup cooperates with the yeast cells for extracting electrons generated via the reduction process of NAD^{+} and accelerates the metabolic activity. Simultaneous generation of electricity and bioethanol production was investigated by using an *S. cerevisiae*-based MFC (in the presence of methylene blue) [296]. This system was fed with glucose as a substrate, which resulted in a stable voltage of approximately 350 mV with 90% ethanol yield (glucose concentration was 20 g/L) in 96 h. Such cogeneration of clean energy and bioethanol production in MFC systems can be the future of fermentation industries as these systems are efficient in utilizing a wide range of substrates [296].

2.8.3.3 Biohydrogen production

Hydrogen in the gaseous stage is considered as carbon-free and promising clean energy source [297], [298]. Anaerobic fermentation of organic substrates by microorganisms is one of the most cost-effective methods for biohydrogen production in hydrogen industries [299]–[301]. The *Cystobasidium slooffiae JSUX1* (an exoelectrogenic yeast strain) was used as an anode biocatalyst in MFC to generate electricity [255]. The electrochemical analysis of *Cystobasidium slooffiae strain JSUX1* revealed that riboflavin secreted by this yeast strain

worked as a mediator that enhanced the electron transfer between cells and the anode. This is the first identified microorganism that simultaneously produced biohydrogen (23 L m^{-3}) and bio-electricity from xylose and generated significant power output of 67 mW m^{-2} [255] .

2.8.3.4 Biosensing

MFC is a device that converts the chemical energy of organic substrates into electricity by biocatalytic actions of electrogenic microorganisms. A conventional MFC biosensor consists of an anaerobic anodic chamber and a cathodic compartment separated by a proton exchange membrane. Microorganisms at the anode oxidize organic substrate and generate electrons and protons. In the cathodic compartment, an oxidation reaction takes place where O_2 reacts with H^+ and produces H_2O, and electrons are passed to the cathode via an external circuit. Thus, current is generated. In yeast-based MFC biosensors, the cathode chamber acts as a sensor probe and it is in direct contact with media spiked with oxygen. The variation in dissolved oxygen (DO) concentration influences the cathode potential. This effects the MFC potential value. The possibility of using MFC based biosensor to monitor biological oxygen demand (BOD) and biotoxicity in an aquatic environment has been validated in past [302], [303]. Different exponential and linear regression models have been evaluated in order to determine the correlation between current density and DO [271]. The results showed that current density increased rapidly with the increase in DO concentration. High DO concentration accelerated the rate of ORR in the cathode chamber [304], [305]. However, the use and application of yeast-based MFC biosensors has been explored scarcely and needs more attention.

2.8.3.5 Wastewater treatment

MFC is an attractive and sustainable option for the conversion of effluent organic matter into bioelectricity. MFCs offer a breakthrough in the simultaneous treatment of organic waste content with energy generation. The efficiency of the two-chamber MFC was studied with *S.*

cerevisiae as an anode biocatalyst [306]. The experimental setup yielded a current density of 994 ± 41 mA m^{-2} and maximum power density of 610 ± 30 mW m^{-2} with 60% substrate concentration, aeration rate of 160 mL/min and pH 6, where sludge has an initial COD concentration in a range of 310–350 mg/L. Volumetric flow rate coupled with COD loading in MFC was 0.18 kg COD/m^3-d. Nevertheless, the energy obtained in yeasts-based MFCs is not higher enough to combat the energy crisis. However, the utilization of fermented sludge in MFCs may handle the issue of fermented broth disposal in industries [306]. In order to enhance the oxygen reduction rate in wastewater collected from a cafeteria, *S. cerevisiae* was used as an anode biocatalyst, while in the cathodic chamber, *Spirulina platensis* (microalgae) worked as an oxygen producer [307]. This combination of yeast and microalgae showed a maximum power density of 98 mW m^{-2} and a maximum current density of 400 mA m^{-2} with simultaneous removal of 82.83% total dissolved solid and 60% COD. Such a combination is immensely useful in yielding a higher quantity of microalgae biomass, which impacts biofuel production [307]. The air cathode MFC with *S. cerevisiae* as an anode biocatalyst was evaluated under various redox conditions and organic loading rates [244]. The power output of yeast-based MFC and wastewater treatment efficiency was evaluated by utilizing synthetic wastewater with a 0.91 kg COD/m^3-d organic loading rate. The maximum current density of 282.83 mA m^{-2} was noted at pH 6.0 with an organic loading rate of 1.43 kg COD/m^3-d, whereas 40.31% of maximum COD removal efficiency was observed at pH 6 with a substrate degradation rate of 0.32 kg COD/m^{-3}-d [244]. In order to reduce COD with enhanced power generation ability, a study evaluated the integration of yeast-based MFC with the liquid fermentation of sugarcane bagasse extract. The authors obtained the maximum power density and COD removal as 14.88 mW m^{-2} and 39.68%, respectively [295].

2.8.3.6 Desalination of water

Microbial desalination cell (MDC) has recently attracted the attention of researchers as a low-cost energy method of water desalination**.** MDC is a variation of MFC, which comprises of a separate compartment (middle chamber) between the anode and cathode [275], [276]. Inside the anode compartment, substrate (organic) is oxidized primarily by microbial activities. Electrons are generated via extracellular electron transfer flow towards the anode and move towards the cathode via an external circuit. The oxygen reduction takes place at the cathode chamber along with protons, which forms water molecules [276], [277]. Generation of H^+ at anode and consumption of H^+ at the cathode initiates desalination of saline water in the middle chamber. Salt ions in the saltwater move across the anion and cation exchange membranes to maintain charge equilibrium [278]**.** Thus, the desalination in the microbial desalination cells does not need any external power input like conventional electrodialysis. This reflects the significant energy benefits of MDC. A new prototype consisting of *S. cerevisiae*-based MFC involving methylene blue as a mediator in anolyte and potassium ferricyanide as catholyte has been investigated [312]. In this study, power production is combined with the desalination of saline water of 0.6 M concentration. The results showed a maximum current density of 88 mA m^{-2} together with 64% removal of total salt (after 30 d). The results proved that yeast-based MFCs are useful in salt removal via an electrically driven membrane process coupled with energy production. Further developments are in progress to enhance power output to make yeast-based MFCs more applicable in saltwater desalination [312].

2.9 Utilization of food waste in MFC

Food waste is a major concern nowadays. It has become a prominent symptom of the agro-food system that requires attention. In recent years, both policymakers and researchers have paid significant attention to the problem of food waste [313]. Due to poverty and the poor functioning of food supply systems, two billion people worldwide are nutrition deficient and

over 800 million people remain hungry every day [314]. On the other hand, yearly 1.3 billion tonnes of food are wasted worldwide, accounting for one-third of global food output [315]. The average per capita food waste for developed counties is 100-170 kg per year which is more than double that in developing countries [14]. This loss not only stretches food production but also inducts environmental issues. Food loss refers to the wastage of food at different stages of the food supply chain, which include production, processing, distribution, retail, and consumption. However, food waste states that losses occur when the food reaches the consumer in the consumption and retail stage, which mainly depends on consumer behaviour [316]. Food industries generate a large amount of food processing waste which include dairy wastes like whey [317], fruit peel/seed waste [318], vegetable peel waste [319], meat and poultry industry waste [320] etc. Most of these wastes include low levels of suspended particles and dissolved solids, which create visual discomfort by producing moldering gases and unpleasant odors and have negative environmental consequences owing to landfill leaching [321].

In order to control this problem, food waste reduction or prevention that avoids the generation of food waste needs to be implemented on a mass scale. It is critical to devise methods for reaching out to small individual caterers and food sectors to establish adequate procedures [322]. Another horizon is resource recovery from food waste. There will always be some losses and wastage throughout the supply chain despite being conscious of it. Food waste comprises of significant amount of carbon-containing components, which include lipids, carbohydrates, amino acids, etc., which can be converted into energy [321].

It has recently been recognized as an underutilized resource with enormous potential for resource recovery. It has a huge potential to be transformed into high-value energy, fuel and natural nutrients by using a variety of approaches [323]. This understanding has inspired fundamental research on technologies that can help in recovering certain valuable resources from food waste. It will reduce the environmental impact of its disposal, avoiding natural

resource depletion, minimizing human health risks and maintaining ecological balance. Food waste is organic waste generated by households, cafeterias, and restaurants, which are responsible for a significant quantity of municipal solid waste. The conventional method like composting, landfill, and incineration of food waste contaminates groundwater and emit toxic gases [324]. Also, the valuable nutrients present in food waste remain unutilized. Biodegradable food waste could be a potential source to recover energy. MFC are effective, safe, clean, and sustainable technology for organic waste degradation/treatment with bioenergy generation. Food waste is a never-ending resource for energy generation using MFC [324]. MFC is a potential anaerobic waste treatment setup where microbes are used at the anode to act as biocatalysts and recover energy from organic waste. As a result, utilizing food waste as a carbon source in MFC for power generation can be an ensuring attempt at food waste management and energy recovery. Characteristics of the organic compounds present in the substrate drastically effect the power generation efficiency of microorganisms [324]. Food waste consists of too many carbon sources like cellulose, sucrose, glucose, protein, fat, lipid, etc. Hence, it is a potential energy source for the electroactive microorganisms for the production of bioelectricity[325]. Hydrolysis of organic matter is an essential step in order to promote substrate degradation mediated by pre-treatment processes like microwave pre-treatment and sonication [324]. Moreover, after the MFC treatment, food waste needs a post-treatment in order to minimize the burden of environmental pollution. Figure 2. **13** shows a generalized view of MFC operation with food waste as a microbial substrate.

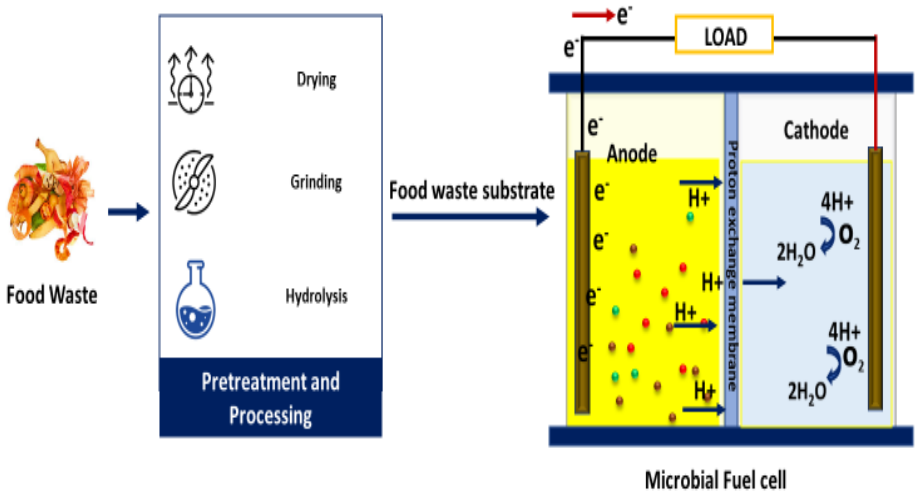

Figure 2. 13. Generalized view of MFC operation with food waste as a microbial substrate.

Table 2. 10 summarise available studies based on power generation using food waste.

Table 2. 10. Bioelectricity generation using food waste MFCs

Substrate	MFC	Inoculum	Anode	Cathode	Power density	Reference
Canteen food waste	Single-chamber air cathode	Anaerobic sludge	Carbon cloth	Carbon cloth	5.6 W/m^3	[324]
Canteen food waste	Single-chamber air cathode	Anaerobic sludge	Graphite plates	Graphite plates	107.89 mW/m^2	[326]
Food waste leachate	Two chamber MFC	No inoculum	Carbon felt	Carbon felt	195.4 ± 18.3 mW/m^3	[327]

Food waste leachate	Two chamber MFC	Domestic wastewater	Carbon felt	Carbon felt	453.90 ± 10.44 mW/m^3	[327]
Food waste leachate	Two chamber MFC	Activated sludge	Carbon felt	Carbon felt	316.12 ± 5.95 mW/m^3	[327]
Food waste leachate	Two chamber MFC	Anaerobic sludge	Carbon felt	Carbon felt	445.61 ± 15.17 mW/m^3	[327]
Canteen food waste	Two chamber MFC	Anaerobic sludge	Graphite	Copper sheet	19151 mW/m^3	[328]
Food waste leachate	Two chamber MFC	Anaerobic sludge	Carbon felt	Carbon felt	657.80 ± 8.34 mW/m^3	[329]
Food waste leachate	Single chamber air-cathode MFC	Anaerobic sludge	Graphite brush	Carbon cloth with Pt catalyst	1540 mW/m^2	[330]
Canteen food waste	Single chamber Solid phase MFC	-	Non-catalysed graphite plates	Non-catalysed graphite plates	170.81 mW/m^2	[331]

Food waste	Single-chamber air cathode MFC	Mixed culture	Graphite felt	Carbon black and polytetrafluoroethylene (PTFE) + 40% Pt/C catalyst	379.4 mW m^{-2}	[332]

Goud et al. (2011) utilized leftover food from the university canteen containing boiled rice, boiled spices, cooked meat, cooked and uncooked rotten vegetables, cooking oil, vegetable peels, and cooked fish [326]. The water content of this food waste was 15 to 24%. The maximum current density of 390 mA/m^2 was observed at an organic loading rate of 1.74 kg COD/m^3-day. Li et al. (2016) utilized food waste collected from the student canteen comprising boiled vegetables, cooked rice, fruits, bones, and cooked meat as the substrate in the MFC. They reported a maximum power density of 5.6 W/m^3 [324]. Li et al. (2013) utilized food waste leachate picked from a leach bed reactor and obtained a maximum power density of 432 mW/m^3. Moharir and Tembhurkar (2018) used kitchen waste (comprising of food and vegetable waste) as a substrate in MFC and obtained a maximum power density of 29.23 mW/m^2 [333]. Besides cooked food waste, fruit processing industries and wholesale fruit/vegetable markets generate giant peel and rotten pulp waste. Fruit processing industries inefficiently manage fruit and vegetable peel waste and are generally discarded in nearby areas. Unmanaged discard of fruit/vegetable waste is the cause of contamination due to its foul smell and becomes the hotspot for houseflies and rodents. Fruit and vegetable peel waste can be an

endless source of energy generation by using MFC. MFC is a potential anaerobic waste treatment setup where microbes used at anode act as biocatalyst and recover energy from organic waste [23]. Table 2. 11 summarise recent work based on power generation using fruit and vegetable waste.

Table 2. 11. Fruit processing waste-based MFCs

Substrate	MFC	Inoculum	Anode	Cathode	Power density	Reference
Citrus peels	Air cathode single chamber MFC	Microflora from anaerobic digester	Plain graphite plate	Plain graphite plate	71.1 mW/m^2	[334]
Blueberry waste	Single-Chamber MFC	*C. boidinii*	Copper	Zinc	3.155 ± 0.24 W/cm^2	[24]
Dried waste mango peels	Dual chamber MFC	*Saccharomyces cerevisiae*	Stainless steel wire mesh	Carbon felt	4.48 mW/m2	[335]
Lime waste	Single-Chamber MFC	-	Copper	Zinc	66 mW/cm2	[22]
Orange waste	Single-Chamber MFC	-	Copper	Zinc	62.5 mW/cm2	[22]
Tangerine waste	Single-Chamber MFC	-	Copper	Zinc	72 mW/cm2	[22]

Corn bran	Membrane-less MFC	Microbes isolated from dewatered sludge	Graphite felt	Graphite felt	12.65 mW m^{-2}	[336]
Banana peel	Membrane-less MFC	Microbes isolated from dewatered sludge	Graphite felt	Graphite felt	23.75 mW m−2	[336]
Orange peel	Dual chamber MFC	Anaerobic consortia	Graphite felt	Platinum-coated graphite cloth	358.8 ± 15.6 mW m^{-2}	[23]
Banana peel	Dual chamber MFC	S. cerevisiae and indigenous microorganisms	Stainless steel mesh	Graphite	86.9 ± 0.4 mW . m^{-2}	[337]
Lemon peel waste	Dual chamber MFC	Anaerobic consortia	Graphite felt	Platinum-coated graphite cloth	371 ± 30 mW m^{-2}	[338]

Fruit peel waste constitutes too many carbon sources like cellulose, sucrose, glucose, fructose, protein, flavonoid, vitamins, etc. Hence, it is the potential energy source for the electrogenic microorganisms for bioelectricity production [336]. Grape waste has also been evaluated as substrate in single chamber MFCs using zinc, copper, magnesium electrodes, thionine and toluidine (red and blue) as mediators. In this case, thionine-based MFC generated

comparatively higher voltages (2.5 V) compared to toluidine red and blue, respectively [339]. MFCs have also been evaluated using papaya waste as substrate, with carbon felt and magnesium oxide as anode and cathode. Papaya based MFC generated power density up to 0.75 to 0.81 mW/cm^2 [340]. As a result, utilizing fruit and vegetable peel waste as a carbon source in MFC for power generation is an ensuring option for food waste management and energy recovery.

2.10 Machine learning

Human beings have the capacity to learn from their past experiences. Similar to human beings, machines can also learn from past experiences or data. Hence, the role of machine learning (ML) has arrived [341]. ML is a branch of artificial intelligence that focuses primarily on developing algorithms that enable a computer to develop its own intelligence from data and prior experiences[341]. ML facilitates systems acquire information from data, optimise performance from experience and anticipate things without being programmed. ML algorithms use sample of earlier data, called "training data," to generate a mathematical model that helps in future prediction and decision making without any explicit programming [342]. Computer science and statistics are brought together by ML to create predictive models. ML generate the algorithms that learn from the past data. Performance of ML algorithms depend on given amount of input dataset. Higher amount of information results the better performance. ML has learning ability and it improve the performance by gaining huge amount of data [342]. **Figure 2. 14** represents block diagram for the working of ML algorithm.

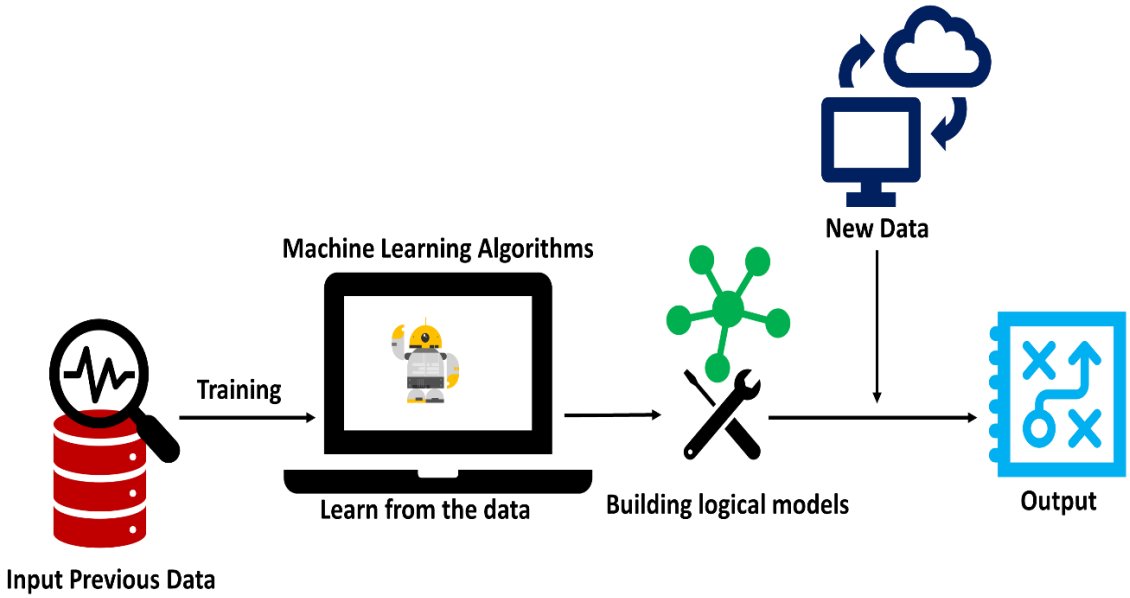

Figure 2. 14. Block diagram of working mechanism of Machine Learning algorithm`

The quantity of data influences the accuracy of projected output since a large amount of data helps to construct a superior model that predicts the output more precisely [343]. Instead of developing code for a difficult matter that requires predictions, just pass the data to generic algorithms, which generate the logic and anticipate the outcome. ML algorithm's learning system can be divided into three segments [343].

2.10.1 Machine learning methods

ML models can be classified into three categories.

2.10.1. 1 Supervised machine learning

Supervised learning, trains computers to categorise data or predict outcomes using labelled datasets. The model modifies its weights to fit input data. Cross validation prevents overfitting and underfitting. Supervised learning helps organizations to solve real challenges. Supervised learning uses naïve bayes, neural networks, support vector machine (SVM), linear regression, random forest and logistic regression [343].

2.10.1. 2 Unsupervised machine learning

Unsupervised learning applies ML algorithms to analyse and cluster unlabelled datasets. These algorithms automatically find hidden patterns and data groupings. This strategy is useful for cross-selling tactics, exploratory data analysis, picture recognition, pattern recognition and consumer segmentation since it can find similarities and contrasts. Unsupervised learning is used to lessen the number of features in a model via dimensionality reduction. Probabilistic clustering, k-means clustering, Neural networks, singular value decomposition (SVD) and principal component analysis (PCA) are the typical methods of unsupervised learning [344].

2.10.1. 3 Semi-supervised learning

Semi-supervised learning blends unsupervised and supervised learning. A smaller labelled data set guides classification and feature extraction from a larger unlabelled data set during training. Semi-supervised learning solves the labelled data issue for supervised learning algorithms. It helps if labelling of enough data is too expensive [344].

2.10.1. 4 Reinforcement learning

Reinforcement learning is an ML model like supervised learning but without sample data. Trial and error strategy is used to train this model. Successful results will reinforce the optimal solution or policy for an issue [344].

2.10.2 ML algorithms

Few different types of ML algorithms are often used. These are the following:

2.10.2.1 Neural networks

Neural networks mimic the arrangements and functionality of the human brain, with a lots of connected processing nodes. Neural networks are good at detecting patterns and are used in

many important ways, such as translating natural language, creating images, recognising images and speech [343].

2.10.2.2 Linear regression

Linear regression is used for predicting numerical values based on the linear relationship of different given data values. For example, the approach may be used to forecast housing values based on previous data of particular geographical area [343].

2.10.2.3 Logistic regression

This supervised learning algorithms generates predictions for categorical response variables, such as "yes/no" responses to queries, and it may be used for applications like the classification of spam and quality control on a manufacturing line [343].

2.10.2.4 Clustering

Using unsupervised learning, clustering algorithms can recognise data patterns in order to classify them. Computers can assist data scientists by spotting discrepancies between data points that human beings have missed [341].

2.10.2.5 Decision trees

Decision trees can be applicable to categorise data and predict numerical values (regression). The usage of decision trees involves constructing a branching arrangement of related decisions, which may be represented graphically using a tree diagram. In contrast to the black box of neural networks, decision trees are straightforward to verify and examine. This is one of the primary benefits of using decision trees [345].

2.10.2.6 Random forests

Random forest algorithm predicts a numerical value or categorical data by combining the output of multiple decision trees to obtain a single output [346].

2.10.3 Decision tree Model

Decision tree is a frequently used ML algorithm that can be applied for both classification and regression task. Decision trees are easy to execute, understand and comprehend which makes this an ideal option for ML applications. A decision tree could potentially assist one in making effective judgements. Decision trees graphically assess options and assign values by merging uncertainties into numerical values [345].

Decision tree is a hierarchically arranged model applicable in decision making and potential outcomes. Decision tree models is non parametric supervised learning and use conditional control statements for decision making. Decision tree models are useful for both regression and classification tasks [347]. Decision tree model build a tree like hierarchical structure which includes a root node, branches, internal nodes and leaf nodes. Figure 2. 15 depicts a general structure of decision tree model.

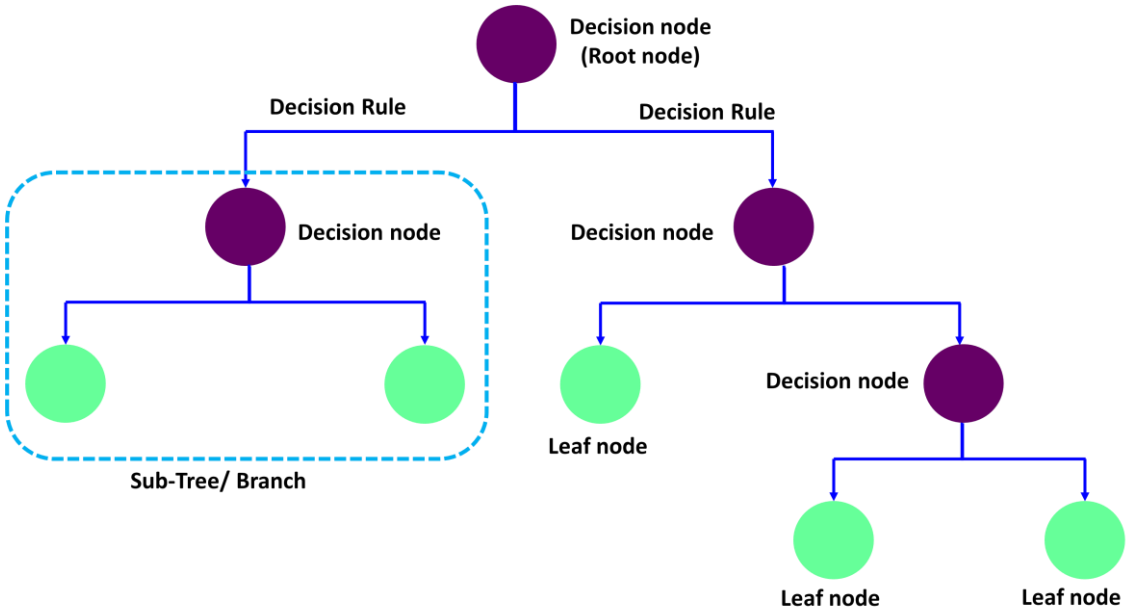

Figure 2. 15. General structure of a decision tree model

Decision tree have some common terminologies as follows:

2.10.3.1 Root Nodes

Root node is present at the top of a decision tree. Root node divides according to different parameters and the population starts divides accordingly [347].

2.10.3.2 Decision Nodes

Decision nodes are the nodes generated after splitting the root nodes [347].

2.10.3.3 Leaf Nodes

Leaf nodes are also known as terminal nodes. At leaf nodes no splitting is possible to further propagate the decision tree [347].

2.10.3.4 Sub-tree

A small portion or the sub-section of the decision tree model is known as a sub-tree [347].

2.10.3.5 Pruning

Pruning is a process of cutting down some nodes to stop overfitting. The accuracy of the decision tree is not compromised in the process of optimising the decision tree by removing irrelevant branches [347].

2.10.3.6 Entropy

Entropy is a measurement that is used in information theory to assess the degree of impureness or uncertainty that exists within a collection of data. It establishes the manner in which a decision tree decides to partition the data [348]. Consider a dataset with N classes. The entropy may be calculated using the formula below:

Consider a dataset with N different classifications. The following expression can be used to calculate the entropy:

$$\text{Entropy}(s) = -\sum_{i=1}^{N} p_i \log_2 p_i \tag{2.3}$$

Where, p_i is the probability of randomly choosing a sample in class i.

2.10.3.7 Information Gain

Information gain can be considered as a measure of how much information a feature delivers regarding a class. Information gain assists to estimate the order of attributes in the decision tree nodes. The principal node is considered as the parent node. Sub nodes are considered as child nodes. Information gain is used to estimate the quality of nodes splitting in a decision tree[348]. The calculation of information gain is as follows:

$$\text{Information gain} = \mathrm{E}_{\mathrm{parant}} - \mathrm{E}_{\mathrm{child}} \tag{2.4}$$

Where, E_{parent} is the entropy of the parent node and E_{child} is the average entropy of the child node.