

MICROBIAL FUEL CELL TECHNOLOGY: A REVIEW ON ELECTRICITY GENERATION

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Abstract: In this review a brief description is given for the biofuel cells along with their classification. The review is mainly intended to concentrate on microbial fuel cells (MFCs) where micro-organisms are used to convert the fuel to electrical energy and also this review gives an overview of the advances made in the past two years in MFCs in terms of their research, development and performance. Some researchers have uncovered the greatest value of MFC technology and which may not be used for the large scale production of electricity, however, they have demonstrated their ability to degrade wastes and toxic chemicals in a greener way. Various approaches on further development to overcome the current challenges in MFCs have been reviewed. This innovative technology will have a major impact and benefit in medical science, clinical research and energy production from renewable sources.

Key words: Microbial fuel cell; Biofuel cells; Enzymatic fuel cell

INTRODUCTION

Biological fuel cells (biofuel cells) are defined as fuel cells those rely on enzymatic catalysis for at least part of their activity [1]. In another words one can define biofuel cells as those cells which uses biocatalysts including systems utilising non-enzyme proteins or biological fuel cells are devices capable of directly transforming chemical to electrical energy via electrochemical reactions involving biochemical pathways. There are two types of biological fuel cells, namely microbial fuel cells and enzymatic fuel cells.

Microbial fuel cells: Microbial fuel cells (MFCs) are devices that can use bacterial metabolism to produce an electrical current from a wide range of organic substrates. The use of microorganisms in biological fuel cells eliminates the isolation of individual enzymes, thereby providing cheaper biocatalysts for biological fuel cells. Microorganisms can be used in four ways for producing electrical energy:

(i) Microorganisms can produce electrochemically

active substances through fermentation or metabolism. For the purpose of energy generation, fuels are produced in separate reactors and transported to the anode of a conventional fuel cell. Accordingly, in this configuration, the microbial bioreactor is kept separated from the fuel cell.

(ii) In the second configuration, the microbiological fermentation process proceeds directly in the anodic compartment of the fuel cell.

(iii) In the case of third configuration, electron-transfer mediators shuttle electrons between the microbial biocatalytic system and the electrode. The mediator molecules accept electrons from the biological electron transport chain of the microorganisms and transport them to the anode of the biological fuel cell.

(iv) In the fourth configuration, the metal-reducing bacterium having cytochromes in its outer membrane and the ability to communicate electrically with the electrode surface directly result in a mediator-less

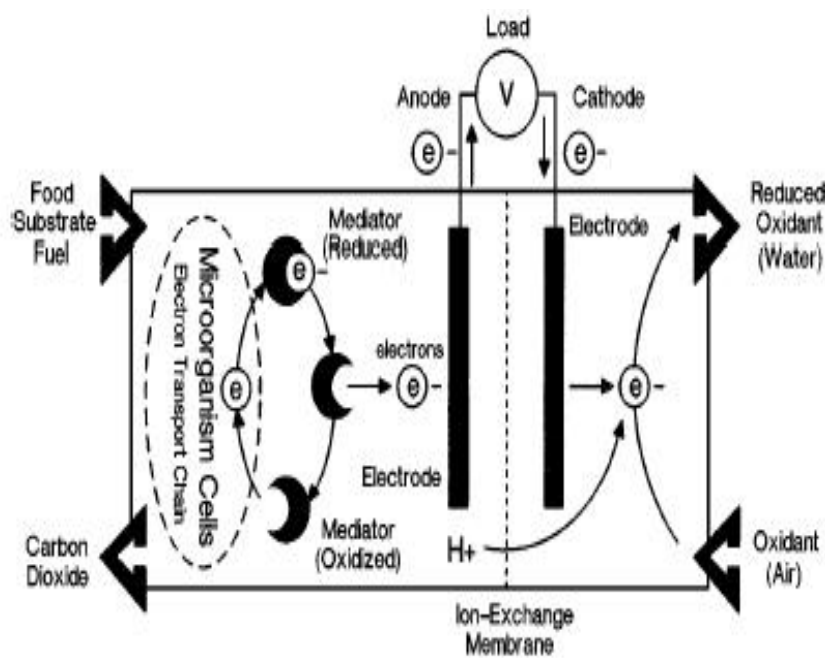


Fig. 1: representing the metabolizing of reactions in anode chamber those are run an- aerobically. An oxidation-reduction mediator diverts electrons from the transport chain. The mediator enters the outer cell lipid membranes and plasma wall, gathers electrons, shuttles them to the anode.

biological fuel cell [1]. Though many types of biofuel cells are developed, a basic model of the biofuel cell is depicted in figure 1.

Enzymatic biofuel cells: These are the devices those use an enzyme to catalyze the fuel oxidation and these can be divided into two categories:

(a) Secondary or indirect enzyme-based biofuel cells: Biocatalyst in a biofuel cell may simply promote the production of simple fuels such as hydrogen or methane from more complicated biochemical substrates such as sugars. Simple fuels are then oxidized by inorganic catalysts at the surface of the electrodes to produce electricity. Biocatalysts mostly prefer ambient temperature whereas metal-catalyzed fuel cell reactions usually require elevated temperature.

(b) Primary or direct biofuel cells: These can be defined as biocatalysts those are directly involved in the redox reaction or reaction chain for the generation of electricity. In this type, enzymes are immobilized on electrodes to facilitate the repeated use of the catalysts. The fuel is enzymatically oxidized at the anode producing protons and electrons. At the cathode, the oxidant (usually oxygen or peroxides) reacts with electrons and protons and then subsequently generates water.

In this contribution we have evaluated very recently published papers and reviews in relation to the microbial fuel cells (MFCs) and their potential applications in the electricity generation. Wang et al. [2] extensively reviewed the performance of microbial fuel cells (MFCs) based on mass transport, reaction kinetics and ohmic resistance. Additional possible applications and future directions are also discussed by these authors for the development of micro-sized MFCs [2].

Separator plays an important role in MFCs. Despite of the rapid development of separators in recent years, there are remaining barriers such as proton transfer limitation and oxygen leakage, which increase the internal resistance and decrease the MFC performance, and thus limit the practical application of MFCs. Li et al. [3] discussed about various separator materials, including cation exchange membrane, anion exchange membrane, bipolar membrane, microfiltration membrane, ultrafiltration membranes, porous fabrics, glass fibers and J-Cloth and salt bridge and systematically compared them. In addition, recent progresses in separator configuration, especially the development of separator electrode assemblies are also summarized. The advances in separator materials and configurations have opened up new promises to overcome the limitations such as cost, performance duration and scaling up problems. Moreover, the authors provided

an outlook for future development and scaling-up of MFC separators [3].

Broad application of MFCs requires low cost and high operational sustainability. Microbial-cathode MFCs or cathodes using only bacterial catalysts (biocathodes) can satisfy these demands and have gained considerable attention in recent years. Achievements with biocathodes in the past have been particularly impressive not only with respect to the biological aspects but also the system-wide considerations related to electrode materials and solution chemistry. The versatility of biocathodes enables one to use not only oxygen but also contaminants as possible electron acceptors, allowing nutrient removal and bioremediation in conjunction with electricity generation. Moreover, biocathodes create opportunities to convert electrical current into microbially generated reduced products. The authors also highlighted the opportunities, limits, and challenges of biocathodes in this contribution [4].

Microbial fuel cells and electricity generation:

Wen et al. [5] demonstrated a single chamber MFC with an air-cathode using glucose–ceftriaxone sodium mixtures or ceftriaxone sodium as fuel. Results showed that the ceftriaxone sodium can be biodegraded and able to produce electricity simultaneously. Interestingly, these ceftriaxone sodium–glucose mixtures played an active role in the production of electricity. The maximum power density is increased in comparison to 1000 mg L⁻¹ glucose (19 W m⁻³) by 495% for 50 mg L⁻¹ ceftriaxone sodium + 1000 mg L⁻¹ glucose (113 W m⁻³), while the maximum power density is 11 W m⁻³ using 50 mg L⁻¹ ceftriaxone sodium as the sole fuel. Moreover, ceftriaxone sodium biodegradation rate reached 91% within 24 h using the MFC in comparison with 51% using the traditional anaerobic reactor. These results indicate that some toxic and bio-refractory organics such as antibiotic wastewater are suitable resources for electricity generation using the MFC technology [5].

Catal et al. [6] examined the direct generation of electricity from a mixture of carbon sources using single chamber mediator-less air cathode MFCs at sub-ambient temperatures. Electricity was directly generated from a carbon source mixture of D-glucose, D-galactose, D-xylose, D-glucuronic acid and sodium acetate at 30 °C and <20 °C (down to 4 °C). Anodic biofilms enriched at different temperatures using

carbon source mixtures were examined using epifluorescent, scanning electron microscopy, and also cyclic voltammetry was used for electrochemical evaluation. The maximum power density obtained at different temperatures ranged from 486 ± 68 mW m⁻² to 602 ± 38 mWm⁻² at the current density range of 0.31 mA cm⁻² to 0.41 mA cm⁻² (14 °C and 30 °C, respectively). Coulombic efficiency increased with decreasing temperature, and ranged from 24 ± 3 to 38 ± 1% (20 °C and 4 °C, respectively). Chemical oxygen demand (COD) removal was over 68% for all carbon sources tested by the authors [6].

The performance of a dual anode-chambered MFC inoculated with *Shewanella oneidensis* MR-1 was evaluated by Kim et al. [7]. This reactor was constructed by incorporating two anode chambers flanking a shared air cathode chamber in an electrically parallel, geometrically stacked arrangement. The device was shown to have the same maximum power density (approximately 24 W m⁻³, normalized by the anode volume) as a single anode and single cathode-chambered MFC. The dual anode-chambered unit generated a maximum current of 3.66 mA (at 50 Ω), twice the value of 1.69 mA (at 100 Ω) for the single anode-chambered device at approximately the same volumetric current density. Increasing the Pt-coated cathode surface area by 100% (12 to 24 cm²) had no significant effect on the power generation of the dual anode-chambered MFC, indicating that the performance of the device was limited by the anode. The medium recirculation rate and substrate concentration in the anode were varied to determine their effect on the anode-limited power density. At the highest recirculation rate of 5 ml min⁻¹, the power density was about 25% higher than at the lowest recirculation rate of 1 ml min⁻¹. The dependence of the power density on the lactate concentration showed saturation kinetics with half-saturation constant K_s in the order of 4.4 mM [7].

Increasing power production and columbic efficiency (CE) of MFCs is a common research ambition as the viability of the technology depends to some extent on the measures of performance. As MFCs are typically time varying systems, comparative studies of controlled and un-controlled external load impedance are needed to show if control affects the biocatalyst development and hence MFC performance. Premier et al. [8], showed the application of logic based control of external load

resistance to increase the power generated by the MFC when compared to an equivalent system which has a static resistive load. The controlled MFC generated 1600 ± 400 °C, compared to 300 ± 10 °C with an otherwise replicate fixed load MFC system. The use of a parsimonious gradient based control was able to increase the CE to within the range of 15.1–22.7%, while the CE for a 200 Ω statically loaded MFC lay in the range 3.3–3.7%. The controlled MFC improves the electrogenic anodic biofilm selection for power production, indicating that greater power and substrate conversion can be achieved by controlling load impedance. Load control ensured sustainable current demand, applied microbial selection pressures and provided near-optimal impedance for power transference, compared to the un-controlled system [8].

To evaluate a suitable electrode material for the efficient green energy generation of a bio-fuel cell, carbonaceous based carbon cloth, carbon paper, and carbon felt electrodes were investigated under different mediators by Ho et al. [9]. The larger surface area, low resistance, and open network of interwoven fibers of the carbon felt electrode facilitated higher electron transfer from the microbial organisms to the electrode surface than that of other carbonaceous electrodes. Carbon paper electrode exhibited lower fuel cell performances due to its lower roughness and high tortuous nature. The green power generation experiments were also carried out by the authors under different mediators such as 2-hydroxy-1,4-naphthoquinone and thionin. The electrons mitigation and power generation was augmented by 2-hydroxy-1,4-naphthoquinone than thionin due to its high solubility, stability and minimal adsorption characteristic to the electrodes. By the combined efforts of extended electrons generation and transportation, bio-fuel cell performances were extended in this work and endorsed its doable applications in bio-fuel cells [9].

Geobacter sulfurreducens is one of the few microorganisms available in pure culture known to directly accept electrons from a negatively poised electrode. Strycharz et al. [10] used microarray analysis to compare gene transcript abundance in biofilms of *G. sulfurreducens* using a graphite electrode as the sole electron donor for fumarate reduction, compared with transcript abundance in biofilms growing on the same material, but not consuming current. Surprisingly, genes for putative

cell-electrode connections, such as outer-surface cytochromes and pili, which are highly expressed in current-producing biofilms, were not highly expressed in current-consuming biofilms. Microarray analysis of *G. sulfurreducens* gene transcript abundance in current-consuming biofilms versus current-producing biofilms gave similar results. In both comparative studies, current-consuming biofilms had greater transcript abundance for a gene (GSU3274) encoding a putative monoheme, *c*-type cytochrome. Deletion of genes for outer-surface proteins previously shown to be essential for optimal electron transfer to electrodes had no impact on electron transfer from electrodes. Deletion of GSU3274 completely inhibited electron transfer from electrodes, but had no impact on electron transfer to electrodes. The differences in gene expression patterns and the impact of gene deletions suggest that the mechanisms for electron transfer from electrodes to *G. sulfurreducens* differ significantly from the mechanisms for electron transfer to the electrodes [10].

Bioelectrochemical systems (BESs) employing mixed microbial communities as biocatalysts are gaining potential importance in renewable energy, bioremediation, or biosensing devices. As it is the beginning to understand how individual microbial species interact with an electrode as electron donor, little is known about the interactions between different microbial species in a community: sugar fermenting bacteria can interact with current producing microbes in a fashion that is either neutral, positively enhancing, or even negatively affecting. Rosenbaum et al. [11], compared the bioelectrochemical performance of *Shewanella oneidensis* in a pure-culture and in a co-culture with the homolactic acid fermenter *Lactococcus lactis* at conditions that are pertinent to conventional BES operation. While *S. oneidensis* alone can only use lactate as electron donor for current production, the co-culture is able to convert glucose into current with a comparable coulombic efficiency of about 17%. By using (electro)-chemical analysis and transcription profiling, the authors found that the BES performance and *S. oneidensis* physiology were not significantly different whether grown as a pure- or co-culture. Thus, the microbes worked together in a purely substrate based (neutral) relationship. These co-culture experiments represent an important step in understanding microbial interactions in BES communities with the goal to design complex microbial communities, which specifically converts target substrates into electricity [11].

Pandit et al. [12] compared the performance of the cathodic electron acceptors (CEA) used in the two-chambered MFC. The performance of the CEA used in the two-chambered MFC was in the following order: potassium permanganate (1.11 V; 116.2 mW/m²) > potassium persulfate (1.10 V; 101.7 mW/m²) > potassium dichromate, K₂Cr₂O₇ (0.76 V; 45.9 mW/m²) > potassium ferricyanide (0.78 V; 40.6 mW/m²). Different operational parameters were considered to find out the performance of the MFC like initial pH in aqueous solutions, concentrations of the electron acceptors, phosphate buffer and aeration. Potassium persulfate was found to be more suitable out of the four electron acceptors which had a higher open circuit potential (OCP) but sustained the voltage for a much longer period than permanganate. Chemical oxygen demand (COD) reduction of 59% was achieved in this work using 10 mM persulfate in a batch process. RALEX™ AEM-PES, an anion exchange membrane (AEM) performed better in terms of power density and OCP in comparison to Nafion®117 Cation Exchange Membrane (CEM) [12].

Tang et al. [13], continuously electrochemically oxidized the graphite felts in order to increase the current generation in MFCs. The treated and untreated graphite felts were utilized as anodes in MFCs and current production was compared. The current production on electrochemically treated graphite felt anodes was about 1.13 mA, 39.5% higher when compared with that of MFCs containing untreated anodes. The results demonstrated that the electronic coupling between graphite felt electrodes and electrogenic bacteria can be enhanced by electrochemical oxidization of the electrodes. Further study showed that the newly generated carboxyl containing functional groups from electrochemical oxidization were responsible for the enhanced electron transfer due to their strong hydrogen bonding with peptide bonds in bacterial cytochromes [13].

Yuan et al. [14] used a simple and efficient electrochemical technique cyclic voltammetry (CV), to quantitatively measure the electron transfer capability of anodic biofilms enriched with acetate and glucose in single-chamber MFCs. Two pairs of distinct redox peaks were observed by CV measurements in both biofilms, identical to the CV features of a pure *Geobacter* strain. The CVs also revealed a higher density of electroactive species in the acetate-enriched biofilm than that of in the glucose-

enriched biofilm. Based on the scan rate analysis, the apparent electron transfer rate constants (k_{app}) in the acetate-enriched biofilm and glucose-enriched biofilm were determined to be 0.82 and 0.15 s⁻¹, respectively, which supported the higher power output of the MFC fed with acetate. Meanwhile, the pH dependence of the biofilms was studied by monitoring the changes of the biofilm redox peak currents and potentials. The authors found that redox reaction of the electrochemical active species in biofilms is pH dependent, and also both electrons and protons involvement in the redox reactions [14].

The ability of electron transfer from microbe cell to anode electrode plays a key role in MFC. Wen et al. [15] explored a new approach to improve the MFC performance and electron transfer rate through addition of Tween 80. Results demonstrated that for an air-cathode MFC operating on 1 g⁻¹ glucose, the addition of Tween 80 increases from 0 to 80 mg L⁻¹, the maximum power density increases from 21.5 to 187 W m⁻³ (0.6–5.2 W m⁻²), the corresponding current density increases from 1.8 to 17 A m⁻², and the resistance of MFC decreases from 27.0 to 5.7 Ω. Electrochemical impedance spectroscopy (EIS) analysis suggested that the improvement of overall performance of the MFC can be attributed to the addition of Tween 80. The high power density achieved here is due to the increase of permeability of cell membranes by addition of Tween 80, which reduces the electron transfer resistance through the cell membrane and increases the electron transfer rate and number, consequently enhances the current and power output. A promising way of utilizing surfactant to improve energy generation of MFC is demonstrated by the authors [15].

Mesh current collectors made of stainless steel (SS) can be integrated into MFC cathodes constructed of a reactive carbon black and Pt catalyst mixture and a poly(dimethylsiloxane) (PDMS) diffusion layer is available in the literature. Zhang et al. [16] showed about the mesh properties of the cathodes and its relation on performance of MFC. Cathodes made from the coarsest mesh (30-mesh) achieved the highest maximum power of 1616 ± 25 mW m⁻² (normalized to cathode projected surface area; 47.1 ± 0.7 W m⁻³ based on liquid volume), while the finest mesh (120-mesh) had the lowest power density (599 ± 57 mW m⁻²). EIS showed that charge transfer and diffusion resistances decreased with increasing mesh opening size. In MFC tests, the cathode

performance was primarily limited by reaction kinetics, and not mass transfer. Oxygen permeability increased with mesh opening size, accounting for the decreased diffusion resistance. At higher current densities, diffusion became a limiting factor, especially for fine mesh with low oxygen transfer coefficients. The results demonstrated the critical nature of the mesh size used for constructing MFC cathodes [16].

One of the challenges in using wireless sensors that require high power to monitor the environment is finding a renewable power source that can produce enough power. Sediment microbial fuel cells (SMFCs) are considered as an alternative renewable power source for remote monitoring. Donovan et al. [17] designed a custom power management system (PMS) to store microbial energy in capacitors and use the stored energy in short bursts. Results demonstrated that SMFCs can be a viable alternative renewable power source for remote sensors that are requiring high power; however, current research on SMFCs has demonstrated that they can only produce several tens of mW of continuous power. This limits the use of SMFCs as an alternative renewable remote power source to mW-level power and this low power is only enough to operate a low-power sensors [17].

The development of highly efficient anode materials is critical for enhancing the current output of microbial electrochemical cells. Fan et al. [18] developed Au and Pd nanoparticle decorated graphite anodes and evaluated in a newly designed multi-anode microbial electrolysis cell (MEC). The anodes decorated with Au nanoparticles produced current densities up to 20-fold higher than plain graphite anodes by *Shewanella oneidensis* MR-1, while those of Pd-decorated anodes with similar morphologies produced 50–150% higher than the control. Significant positive linear regression was obtained between the current density and the particle size (average Feret's diameter and average area), while the circularity of the particles showed negative correlation with current densities. On the contrary, no significant correlation was evident between the current density and the particle density based on area fraction and particle counts. The results demonstrated that nano-decoration can greatly enhance the performance of microbial anodes, while the chemical composition, size and shape of the nanoparticles determine the extent of the enhancement [18].

MFCs have generated excitement in environmental and bioenergy communities due to their potential for

coupling wastewater treatment with energy generation and powering diverse devices. The pursuit of strategies such as improving microbial cultivation practices and optimizing MFC devices has increased power generating capacities of MFCs. However, surprisingly few microbial species with electrochemical activity in MFCs have been identified because current devices do not support parallel analyses or high throughput screening. Hou et al. [19] extended their earlier study of fabrication of a MFC microarray using advanced microfabrication methods by demonstrating a microfabricated air-cathode MFC array system. The system contained 24 individual air-cathode MFCs integrated onto a single chip. The device enabled the direct and parallel comparison of different microbes loaded onto the array. Environmental samples were used to validate the utility of the air-cathode MFC array system and two previously identified isolates, 7Ca (*Shewanella* sp.) and 3C (*Arthrobacter* sp.) were shown to display enhanced electrochemical activities of 2.69 mW/m² and 1.86 mW/m², respectively. Experiments using a large scale conventional air-cathode MFC validated these findings. The parallel air-cathode MFC array system demonstrated here is expected to promote and accelerate the discovery in addition to the characterization of electrochemically active microbes [19].

Biocathode MFCs are of great potentials in bioremediation of Cr(VI)-contaminant sites due to their low operating cost, self-regenerating ability and sustainable power supply. The improvement of Cr(VI) reduction rates and power generation, however, remains to be a challenging. Huang et al. [20] evaluated graphite fibers, graphite felt and graphite granules as biocatalytic cathode materials in tubular MFCs in terms of Cr(VI) reduction and electricity generation. At cathode to anode surface area ratio (C/A) of 3, graphite fiber was found superior to graphite felt or graphite granule. Specific Cr(VI) reduction rates ranging from 12.4 to 20.6 mg g⁻¹ VSS h⁻¹ and power generation from 6.8 to 15 W m⁻³ (20–48 A m⁻³) were achieved in the biocatalytic graphite fiber cathode MFCs. Under a temperature of 22 °C and pH 7.0, Cr(VI) reduction followed pseudo-first-order kinetic model with the rate constant being 0.451 ± 0.003 h⁻¹. In comparison with pH 7.0, an acidic pH of 5.0 improved Cr(VI) reduction to 27.3% and power generation to 61.8% whereas an alkaline pH of 8.0 decreased Cr(VI) reduction to 21.2% and power generation to 6.0%. It was found in this study that the products formed

whether as dissolved and/or precipitated Cr(III) was heavily pH dependent. Elevating temperature from 22 to 50 °C increased Cr(VI) reduction with the apparent activation energy (E_a) obtained as 10.6 kJ mol⁻¹. At high temperature of 50 °C, however, a decreased power generation was observed mainly because of the increase in anode potential. The results indicated that an optimal condition exists for efficient biocathode MFCs with quick Cr(VI) reduction and simultaneous high power generation [20].

MFCs are reactors and able to generate electricity by capturing electrons from the anaerobic respiratory processes of microorganisms. While the majority of MFCs have been tested at ambient or mesophilic temperatures, thermophilic systems warrant evaluation because of the potential for increased microbial activity rates on the anode. MFC studies at elevated temperatures have been scattered using designs that are already established, specifically air-cathode single chambers and two-chamber designs. Carver et al. [21] described a thermophilic MFC design that prevents evaporation. The design was tested at 57 °C with an anaerobic, thermophilic consortium that respired with glucose to generate a power density of 375 mW m⁻² after 590 hrs. Polarization and voltage data showed the working capability of the design in the batch mode and also design allows for adaption to continuous operation [21].

Scaling up of microbial fuel cells (MFCs) requires a better understanding of the importance of the different factors such as electrode surface area and reactor geometry relative to solution conditions such as conductivity and substrate concentration. Cheng et al. [22] showed that the substrate concentration has significant effect on anode but not on cathode performance, while the solution conductivity has a significant effect on the cathode but not on the anode. The cathode surface area is always important for increasing power. Doubling the cathode size can increase power by 62% with domestic wastewater, but doubling the anode size increases power by 12%. Volumetric power density was shown to be a linear function of cathode specific surface area (ratio of cathode surface area to reactor volume), but the impact of cathode size on power generation depended on the substrate strength (COD) and conductivity. The results indicated that the cathode specific surface area is the most critical factor for scaling-up of MFCs to obtain high power densities [22].

External resistance affects the performance of MFCs by controlling the flow of electrons from the anode to the cathode. Yazdi et al. [23] determined the effect of external resistance on bacterial diversity and metabolism in MFCs. Four external resistances (20, 249, 480, and 1000 Ω) were tested by operating parallel MFCs independently at constant circuit loads for 10 weeks. A maximum power density of 66 mW m⁻² was achieved by the 20 Ω MFCs, while the MFCs with 249, 480, and 1000 Ω external resistances produced 57.5, 27, and 47 mW m⁻², respectively. Denaturing gradient gel electrophoresis analysis of partial 16S rRNA genes showed clear differences between the planktonic and anode-attached populations at various external resistances. Concentrations of short chain fatty acids were higher in MFCs with larger circuit loads, suggesting that fermentative metabolism dominated over anaerobic respiration using the anode as the final electron acceptor [23].

Zhuang et al. [24] described an *Enterobacter aerogenes*-catalyzed MFC with a carbon-based anode that exhibited a maximum power density of 2.51 W/m³ in the absence of artificial electron mediators. The MFC started up rapidly, within hours, and the current generation in the early stage was demonstrated to result from *in situ* oxidation of biohydrogen produced by *E. aerogenes* during glucose fermentation. Over periodic replacement of substrate, both planktonic biomass in the culture liquid and hydrogen productivity decreased, while increased power density and coulombic efficiency and decreased internal resistance were unexpectedly observed. Using scanning electron microscopy and cyclic voltammetry, it was found that the enhanced MFC performance was associated with the development of electroactive biofilm on the anodic surface, proposed to involve an acclimation and selection process of *E. aerogenes* cells under electrochemical tension. The significant advantage of rapid start-up and the ability to develop an electroactive biofilm identifies *E. aerogenes* as a suitable biocatalyst for MFC applications were discussed [24].

Shewanella-containing MFCs typically use the fresh water wild-type strain *Shewanella oneidensis* MR-1 due to its metabolic diversity and facultative oxidant tolerance. However, *S. oneidensis* MR-1 is not capable of metabolizing polysaccharides for extracellular electron transfer. Biffinger et al. [25]

analyzed the applicability of *Shewanella japonica* (an agar-lytic *Shewanella* strain) for power applications using a diverse array of carbon sources for current generation from MFCs, cellular physiological responses at an electrode surface, biofilm formation, and the presence of soluble extracellular mediators for electron transfer to carbon electrodes. Critically, air-exposed *S. japonica* utilized biosynthesized extracellular mediators for electron transfer to carbon electrodes with sucrose as the sole carbon source [25].

Choi et al. [26] examined the effects of biofouling on the electrochemical properties of cation exchange membranes (CEMs), such as membrane electrical resistance (MER), specific proton conductivity (SC), and ion transport number (t_+), in addition to on the microbial fuel cell (MFC) performance. CEM biofouling using a 15.5 ± 4.6 μm biofilm was found to slightly increase the MER from $15.65 \Omega \text{ cm}^2$ (fresh Nafion) to $19.1 \Omega \text{ cm}^2$, whereas an increase of almost two times was achieved when the electrolyte was changed from deionized water to an anolyte containing a high cation concentration supporting bacterial growth. The simple physical cleaning of CEMs had little effect on the Coulombic efficiency (CE), whereas replacing a biofouled CEM with new one resulted in considerable increase of up to 59.3%, compared to 45.1% for a biofouled membrane. The above results clearly suggest that the internal resistance increase of MFC was mainly caused by the sulfonate functional groups of CEM being occupied with cations contained in the anolyte, rather than biofouling itself [26].

Finch et al. [27] used MFCs to monitor metabolism changes in *Clostridium acetobutylicum* fermentations. When MFCs were inoculated with *C. acetobutylicum*, they generated a unique voltage output pattern where two distinct voltage peaks occurred over a weeklong period. This result was markedly different to previously studied organisms which usually generate one sustained voltage peak. Analysis of the fermentation products indicated dual voltage peaks correlated to glucose metabolism. The first voltage peak correlated with acidogenic metabolism (acetate and butyrate production) and the second peak with solventogenic metabolism (acetone and butanol production). This demonstrates that MFCs can be applied as a novel tool to monitor the shift from acid to solvent production in *C. acetobutylicum* [27].

Viridis et al. [28] showed about the aeration of the cathode compartment of bioelectrochemical systems (BESs) to promote simultaneous nitrification and denitrification (SND). This study investigated the cathodic metabolism under different operating conditions as well as the structural organization of the cathodic biofilm during SND. Results showed a maximal nitrogen removal efficiency of $86.9 \pm 0.5\%$, and a removal rate of $3.39 \pm 0.08 \text{ mg N L}^{-1} \text{ h}^{-1}$ at a dissolved oxygen (DO) level of $5.73 \pm 0.03 \text{ mg L}^{-1}$ in the catholyte. The DO levels used in this study are higher than the thresholds previously reported as detrimental for denitrification. Analysis of the cathodic half-cell potential during batch tests suggested the existence of an oxygen gradient within the biofilm while performing SND. FISH analysis corroborated this finding revealing that the structure of the biofilm included an outer layer occupied by putative nitrifying organisms and an inner layer, where putative denitrifying organisms were most dominant [28].

Chung et al. [29] operated a two-chamber MFC system continuously for more than 500 days to evaluate effects of biofilm and chemical scale formation on the cathode electrode on power generation. A stable power density of 0.57 W/m^2 was attained after 200 days operation. However, the power density decreased drastically to 0.2 W/m^2 after the cathodic biofilm and chemical scale were removed. As the cathodic biofilm and chemical scale partially accumulated on the cathode, the power density gradually recovered with time. Microbial community structure of the cathodic biofilm was analyzed based on 16S rRNA clone libraries. The clones closely related to *Xanthomonadaceae* bacterium and *Xanthomonas* sp. in the *Gammaproteobacteria* subdivision were most frequently retrieved from the cathodic biofilm. Results of the SEM-EDX analysis revealed that the cation species (Na^+ and Ca^{2+}) were main constituents of chemical scale, indicating that these cations diffused from the anode chamber through the Nafion membrane. However, an excess accumulation of the biofilm and chemical scale on the cathode exhibited adverse effects on the power generation due to a decrease in the active cathode surface area and an increase in diffusion resistance for oxygen. In view of the above, it is important to properly control the formation of chemical scale and biofilm on the cathode during long-term operation [29].

Cheng et al. [30] used an inoculum from a two-chamber aqueous–cathode microbial electrolysis cell (MEC) in order to achieved higher power densities in air–cathode MFCs. Air–cathode MFCs with this inoculum produced maximum power densities of 1070 mW m^{-2} (cathode surface area) in single-chamber and 880 mW m^{-2} in two-chamber MFCs. Coulombic efficiencies ranged from 25% to 50%, and COD removals were 50–70% based on total cellulose removals of 60–80%. Decreasing the reactor volume from 26 to 14 mL (while maintaining constant electrode spacing) decreased power output by 66% (from 526 to 180 mW m^{-2}) due to a reduction in total mass of cellulose added. These results demonstrate that air–cathode MFCs can produce high power densities with cellulose following proper acclimation of the inoculum, and that organic loading rates are important for maximizing power densities from particulate substrates [30].

Zhang et al. [31] reported about the combined use of brush anodes and glass fiber (GF1) separators, and plastic mesh supporters to create a scalable MFC architecture. Separators prevented short circuiting of closely-spaced electrodes, and cathode supporters were used to avoid water gaps between the separator and cathode that can reduce power production. The maximum power density with a separator and supporter and a single cathode was $75 \pm 1 \text{ W/m}^3$. Removing the separator decreased power by 8%. Adding a second cathode increased power to $154 \pm 1 \text{ W/m}^3$. Current was increased by connecting two MFCs in parallel. These results show that brush anodes combined with a glass fiber separator and a plastic mesh supporter produce a useful MFC architecture that is inherently scalable due to good insulation between the electrodes and a compact architecture [31].

Conditions in MFCs differ from those in microbial electrolysis cells (MECs) due to the intrusion of oxygen through the cathode and the release of H_2 gas into solution and this was demonstrated by Kiely et al, [32]. Based on 16S rRNA gene clone libraries, anode communities in reactors fed acetic acid decreased in species richness and diversity, and increased in numbers of *Geobacter sulfurreducens*, when reactors were shifted from MFCs to MECs. With a complex source of organic matter (potato wastewater), the proportion of *Geobacteraceae* remained constant when MFCs were converted into MECs, but the percentage of clones belonging to *G.*

sulfurreducens decreased and the percentage of *G. metallireducens* clones increased. A dairy manure wastewater-fed MFC produced little power and had more diverse microbial communities, but did not generate current in an MEC. These results showed changes in *Geobacter* species in response to the MEC environment and the higher species diversity is not correlated with current [32].

Modification of carbon cloth anodes were carried out with 4(*N,N*-dimethylamino)benzene diazonium tetrafluoroborate by Saito et al. [33] to increase nitrogen-containing functional groups at the anode surface in order to test whether the performance of MFCs could be improved by controllably modifying the anode surface chemistry. Anodes with the lowest extent of functionalization, based on a nitrogen/carbon ratio of 0.7 as measured by XPS, achieved the highest power density of 938 mW/m^2 . This power density was 24% greater than an untreated anode, and similar to that obtained with an ammonia gas treatment previously shown to increase power. Increasing the nitrogen/carbon ratio to 3.8, however, decreased the power density to 707 mW/m^2 . These results indicated that a small amount of nitrogen functionalization on the carbon cloth material is sufficient to enhance MFC performance, likely as a result of promoting bacterial adhesion to the surface without adversely affecting microbial viability or electron transfer to the surface [33].

Production of electricity from samples obtained during anaerobic digestion of grass silage was examined by Catal et al. [34] using single-chamber air-cathode mediator-less MFCs. The samples were obtained from anaerobic reactors at start-up conditions after 3 and 10 days of operation under psychrophilic ($15 \text{ }^\circ\text{C}$) and mesophilic ($37 \text{ }^\circ\text{C}$) temperatures. Electricity was directly produced from all samples at a concentration of $1500 \text{ mg COD L}^{-1}$. Power density obtained from the samples, as a sole carbon source, ranged from $56 \pm 3 \text{ W m}^{-3}$ to $31 \pm 1 \text{ W m}^{-3}$ for the mesophilic and psychrophilic samples, respectively. Coulombic efficiencies ranged from $18 \pm 1\%$ to $12 \pm 1\%$ for the same samples. The relationship between the maximum voltage output and initial COD concentration appeared to follow saturation kinetics at the external resistance of $217 \text{ } \Omega$. Chemical oxygen demand (COD) removal was over 90% and total phenolics removal was in the range of 30–75% for all samples tested with a standard amount of 60 mg L^{-1} total phenolics removed for every sample. The results

reveal that generating electricity from solution samples of anaerobic reactors utilizing grass silage is possible, opening the possibility for combination of anaerobic digestion with MFC technology for energy generation [34].

Biodiesel production through transesterification of lipids generates large quantity of biodiesel waste (BW) containing mainly glycerin. BW can be treated in various ways including distillation to produce glycerin used as substrate for fermentative propanediol production and discharge as wastes. In this study Feng et al. [35] examined MFCs to treat BW with simultaneous electricity generation. The maximum power density using BW was 487 ± 28 mW/m² cathode (1.5 A/m² cathode) with 50 mM phosphate buffer solution (PBS) as the electrolyte, which was comparable with 533 ± 14 mW/m² cathode obtained from MFCs fed with glycerin medium (COD 1400 mg/L). The power density increased from 778 ± 67 mW/m² cathode using carbon cloth to 1310 ± 15 mW/m² cathode using carbon brush as anode in 200 mM PBS electrolyte. The power density was further increased to 2110 ± 68 mW/m² cathode using the heat-treated carbon brush anode. Coulombic efficiencies (CEs) increased from $8.8 \pm 0.6\%$ with carbon cloth anode to $10.4 \pm 0.9\%$ and $18.7 \pm 0.9\%$ with carbon brush anode and heat-treated carbon brush anode, respectively [35].

Weld and Singh [36] combined a thermophilic anaerobic digester (AD) with a MFC to evaluate whether either component had increased stability when operated in combination as a hybrid system, perturbed by the addition of acetic acid. The MFC and the anaerobic digester were able to operate effectively together. The MFC was more susceptible to high acetic acid load than the AD. The hybrid system did not increase resilience compared to the solitary systems in the conditions tested. However, the low pH had a relatively delayed effect on the MFC compared to the AD, allowing the hybrid system to have a more stable energy output. Also, at very low pH, when operating as a hybrid, the AD component was able to recover pH to normal levels when the MFC component failed. The outcome of the study indicates that there are synergies that can be gained from this hybrid system [36].

Ren et al. [37] discussed about the relationship between anode microbial characteristics and

electrochemical parameters in MFCs by time-course sampling of parallel single-bottle MFCs operated under identical conditions. While voltage stabilized within 4 days, anode biofilms continued growing during the six-week operation. Viable cell density increased asymptotically but membrane-compromised cells accumulated steadily from only 9% of total cells on day 3 to 52% at 6 weeks. Electrochemical performance followed the viable cell trend with a positive correlation for power density and an inverse correlation for anode charge transfer resistance. The biofilm architecture shifted from rod-shaped, dispersed cells to more filamentous structures with the continuous detection of *Geobacter sulfurreducens*-like 16S rRNA fragments throughout operation and the emergence of a community member related to a known phenazine-producing *Pseudomonas* species. A drop in cathode open circuit potential between weeks two and three suggested that uncontrolled biofilm growth on the cathode deleteriously affects system performance [37].

Surface modifications of anode materials are important for enhancing power generation of MFC. Zhu et al. [38] constructed membrane free single-chamber air-cathode MFCs, MFC-A and MFC-N using activated carbon fiber felt (ACF) anodes treated by nitric acid and ethylenediamine (EDA), respectively. Experimental results showed that the start-up time to achieve the maximum voltages for the MFC-A and MFC-N was shortened by 45% and 51%, respectively as compared to that for MFC-AT equipped with an unmodified anode. Moreover, the power output of MFCs with modified anodes was significantly improved. In comparison with MFC-AT which had a maximum power density of 1304 mW/m², the MFC-N achieved a maximum power density of 1641 mW/m². The nitric acid-treated anode in MFC-A increased the power density by 58% reaching 2066 mW/m². XPS analysis of the treated and untreated anode materials indicated that the power enhancement was attributable to the changes of surface functional groups [38].

The Fe(III)/Fe(II) couple can play a significant role in the abiotic reduction of 2-nitrophenol (2-NP) at the cathode chamber of a MFC. Feng et al. [39] demonstrated that Fe(II) addition to the cathode chamber contributes to a significant increase in the reaction rate of 2-NP removal and the power performance of MFC. The authors observed pseudo

first-order rate constants and power densities which are heavily dependent on the identity of the Fe(II)-complexing ligands. The Fe(II) complex coordinated with citrate results in the highest rate constant up to 0.12 h^{-1} as compared to other organically complexed iron species including Fe(II)-EDTA, Fe(II)-acetate and Fe(II)-oxalate, and iron species uncomplexed with any organic ligands. In addition, the presence of Fe(II)-citrate species leads to a maximum volumetric power density of 1.0 W m^{-3} , which is the highest value among those obtained with other iron species for the similar MFC system [39].

Zhang et al. [40] investigated the effect of substrate changes on the performance and microbial community of two-chamber MFCs. The MFCs enriched with a single substrate (e.g., acetate, glucose, or butyrate) had different acclimatization capability to substrate changes. The MFC enriched with glucose showed rapid and higher power generation, when glucose was switched with acetate or butyrate. However, the MFC enriched with acetate needed a longer adaptation time for utilizing glucose. Microbial community was also changed when the substrate was changed. *Clostridium* and *Bacilli* of phylum *Firmicutes* were detected in acetate-enriched MFCs after switching to glucose. In contrast, *Firmicutes* completely disappeared and *Geobacter*-like species were specifically enriched in glucose-enriched MFCs after feeding acetate to the reactor. This study further suggests that the type of substrate fed to MFC is a very important parameter for reactor performance and microbial community, and significantly affects power generation in MFCs [40].

Watson and Logan [41] discussed about the polarization curves from MFCs that often show an unexpectedly large drop in voltage with increased current densities leading to a phenomenon in the power density curve referred to as “power overshoot”. Linear sweep voltammetry (LSV, 1 mV s^{-1}) and variable external resistances (at fixed intervals of 20 min) over a single fed-batch cycle in an MFC both resulted in power overshoot in power density curves due to anode potentials. Increasing the anode enrichment time from 30 days to 100 days did not eliminate overshoot, suggesting that insufficient enrichment of the anode biofilm was not the primary cause. Running the reactor at a fixed resistance for a full fed-batch cycle (~ 1 to 2 days), however, completely eliminated the overshoot in the power density curve. These results showed that long

times at a fixed resistance are needed to stabilize current generation by bacteria in MFCs, and that even relatively slow LSV scan rates and long times between switching circuit loads during a fed-batch cycle may produce inaccurate polarization and power density results for these biological systems [41].

Sun et al. [42] designed and demonstrated a MFC incorporating a newly developed aerobic biocathode. The aerobic biocathode MFC is able to further treat the liquid containing decolorization products of active brilliant red X-3B (ABRX3) a respective azo dye and also provided increased power production. Batch test results showed that 24.8% of COD was removed from the decolorization liquid of ABRX3 (DL) by the biocathode within 12 h. Metabolism-dependent biodegradation of aniline-like compound might be mainly responsible for the decrease of overall COD. Glucose is not necessary in this process and contributes little to the COD removal of the DL. The similar COD removal rate observed under closed circuit condition (500Ω) and opened circuit condition indicated that the current had an insignificant effect on the degradation of the DL. Addition of the DL to the biocathode resulted in an almost 150% increase in open cycle potential (OCP) of the cathode accompanied by a 73% increase in stable voltage output from 0.33 V to 0.57 V and a 300% increase in maximum power density from 50.74 mW/m^2 to 213.93 mW/m^2 . Cyclic voltammetry indicated that the decolorization products of the ABRX3 contained in the DL play a role as redox mediator for facilitating electron transfer from the cathode to the oxygen [42].

The interface resistances between an anion exchange membrane (AEM) and the solution electrolyte were measured by Ji et al. [43] for low buffer (or ionic strength) electrolytes based typical MFCs. Three AEMs (AFN, AM-1, and ACS) having different properties were tested in a flat-plate MFC to which 5-mM acetate was fed to the anode and an air-saturated phosphate buffer (PB) solution was fed to the cathode. Current density achieved in the MFCs was correlated inversely with independently measured membrane-only resistances. However, the total interfacial resistances measured by current-voltage plots were approximately two orders higher than those of the membrane-only resistances although membranes had the same order as with the membrane-only resistance. EIS spectra showed that the resistances from electric-double layer and diffusion boundary layer were the main resistances

and not the membrane's resistance. The electric-double layer and diffusion boundary layer resistances of the AEMs were much larger in the 10 mM PB electrolyte, compared to 100 mM PB. EIS study also showed that the resistance of diffusion boundary layer decreased due to mechanical stirring. Therefore, the interface resistance that originates from the interaction between the membrane and the catholyte solution should be considered when designing and operating MFC processes with an AEM. The AEMs allowed transport of uncharged O_2 and acetate, but the current losses for both were low during normal MFC operation [43].

Kuntke et al. [44] investigated ammonium recovery using a two chamber MFC at high ammonium concentration. Increasing the ammonium concentration (from 0.07 g to 4 g ammonium-nitrogen/L) by addition of ammonium chloride did not affect the performance of the MFC. The obtained current densities by DC-voltammetry were higher than 6 A/m² for both operated MFCs. Also continuous operation at lower external resistance (250 Ω) showed an increased current density (0.9 A/m²). Effective ammonium recovery was achieved by migrational ion flux through the cation exchange membrane to the cathode chamber driven by the electron production from degradation of organic substrate. The charge transport was proportional to the concentration of ions. Nonetheless, a concentration gradient influenced the charge transport. Furthermore, a charge exchange process also influenced the charge transport and therefore the recovery of specific ions was attained [44].

Different microfiltration membrane (MFM), proton exchange membrane (PEM) and ultrafiltration membranes (UFMs) with different molecular cut off weights of 1K (UFM-1K), 5K (UFM-5K) and 10K (UFM-10K) were incorporated by Hou et al. [45] into air-cathode single-chamber MFCs which were explored for simultaneous azo dye decolorization and electricity generation to investigate the effect of membrane on the performance of the MFC. Batch test results showed that the MFC with an UFM-1K produced the highest power density of 324 mW/m² coupled with an enhanced coulombic efficiency compared to MFM. The MFC with UFM-10K achieved the fastest decolorization rate (4.77 mg/L h), followed by MFM (3.61 mg/L h), UFM-5K (2.38 mg/L h), UFM-1K (2.02 mg/L h) and PEM (1.72 mg/L h). These results demonstrated the possibility

of using various membranes in the system described in this study and revealed that UFM-1K was the best one based on the consideration of both cost and performance [45].

Katuri and Scott [46] designed a study for the dynamic response of a MFC using membrane electrode assemblies (MEAs) for air breathing cathode operation. The MFC used four MEAs simultaneously and had a low internal resistance. An increased concentration of glucose produced a non-linear increase in the maximum current. The time to reach the maximum current increased with increasing glucose concentrations of 1 to 7 mM; varying from approximately 2.4 to 4.2 hrs. The rate at which the current density increased with time was the same for all glucose concentrations up to current densities close to the maximum values. The peak power density varied approximately linearly with glucose concentrations from 2 to 77 mW/m² (1 to 7 mM) with a 1 k Ω resistance. The cell response appeared to be linked to a slow process of fuel transport to the bacteria and their metabolic processes. The dynamic response of the anode was analysed in terms of a substrate mass transport model. The application of different current ranges did not significantly change the dynamic response of either the anode community or the MFC polarization characteristics. Thus, it is likely that the bacterial communities that form under MFC operation contain sufficiently "dominant" electro-active species that are capable of producing high power for MFCs [46].

Glucose-fed MFCs have displayed low Coulombic efficiency (CE); one reason for a low CE is metabolite generation causing significant electron loss within MFC systems. A notable electron loss (15.83%) was observed by Kim et al. [47] in glucose-fed MFCs due to residual propionate, a glucose metabolite. In order to enhance the low CE caused by metabolite generation, a dual-anode MFC (DAMFC) was constructed, which are separately enriched by dissimilar substrates (glucose and propionate, respectively) to effectively utilize both glucose and propionate in one-anode chamber. In the DAMFC, propionate ceases to exist as a source of electron loss and thus the CE increased from 33 ± 6 to $59 \pm 4\%$ [47].

The internal resistance including activation loss internal resistance (AIR), ohmic loss internal resistance (OIR) and concentration loss internal

resistance (CIR) is an important parameter that determines the performance of MFCs were studied by Zhang et al. [48]. The authors completed the experimental investigations to estimate the contributions of these three components to the internal resistance. The internal resistance was found to vary with electric current, although it was almost a constant for the current was within a certain region. The largest component of the internal resistance was CIR except for small currents. The AIR decreases quickly for small current and reduces its decreasing rate as the current increases and approaches to a constant. The OIR was constant over the whole current range. The experiments also disclosed that increasing the limiting current and reducing the concentration loss are both important for improving the MFC performance [48].

Carbon nanotube (CNT) is a promising electrode material and was used as an anode modifier in MFCs by Liang et al. [49]. Authors used a new method of simultaneously adding CNT powders and *Geobacter sulfurreducens* into the anode chamber of a MFC, aiming to form a composite biofilm on the anode. The performance of MFCs such as startup time and steady-state power generation was investigated under conditions of different CNT powders dosages. Results showed reduced startup time and the anodic resistance. The optimal dosage of CNT powders pre-treated by acid was 4 mg/mL for the anode chamber with an effective volume of 25 mL. The anodic resistance and output voltage of the MFC with CNT powders addition were maintained around 180 Ω and 650 mV during 40 days operation, while those of the MFC without CNT powders addition increased from 250 Ω to 540 Ω and decreased from 630 mV to 540 mV, respectively, demonstrating that adding CNT powders helped to stabilize the anodic resistance, thus the internal resistance and power generation during long-term operation. Based on cyclic voltammogram, the electrochemical activity of anodic biofilm was found to enhance by adding CNT powders, though no significant increase of the biomass in anodic biofilm was detected by phospholipids analysis. There was no remarkable change of ohmic resistance with the addition of CNT powders as revealed by current interrupt method, which indicated that the rate of mass transfer might be promoted by the presence of CNT powders [49].

A two-chamber MFC was reported Nien et al. [50] in the literature using iron-reducing strains as inoculum and acetate as carbon sources. The tested

MFC had an open-circuit voltage of 0.67 V, and reached 1045 mA m⁻² and a power density of 486 mW m⁻² at 0.46 V before power overshoot occurred. Anodic reactions were identified as the rate-determining steps. Stirring of the anolyte insignificantly increased cell performance, suggesting a minimal external mass transfer resistance from the anolyte to the anodic biofilm. Data regression analysis indicated the charge transfer resistance at the biofilm-anode junction and was also negligible. The order of magnitude estimation of electrical conductance indicates that electron transfer resistance had an insignificant effect on MFC performance. Resistance in electrogens for substrate utilization was responsible to induce MFC power overshoot [50].

Currently available models describing MFC polarization curves do not describe the effect of the presence of toxic components. A bioelectrochemical model combined with enzyme inhibition kinetics, that describes the polarization curve of an MFC-based biosensor, was modified to describe four types of toxicity by Stein et al. [51]. To get a stable and sensitive sensor, the overpotential has to be controlled. Simulations with the four modified models were performed to predict the overpotential that gives the most sensitive sensor. These simulations were based on data and parameter values from experimental results under non-toxic conditions. Given the parameter values from experimental results, controlling the overpotential at 250 mV leads to a sensor that is most sensitive to components that influence the whole bacterial metabolism or that influence the substrate affinity constant (K_m). Controlling the overpotential at 105 mV is the most sensitive setting for components influencing the ratio of biochemical over electrochemical reaction rate constants (K_1), while an overpotential of 76 mV gives the most sensitive setting for components that influence the ratio of the forward over backward biochemical rate constants (K_2). The sensitivity of the biosensor was also analyzed for robustness against changes in the model parameters other than toxicity. As an example, the tradeoff between sensitivity and robustness for the model describing changes on K_1 (IK_1) was presented. The biosensor was sensitive for toxic components and robust for changes in model parameter K_2 when overpotential is controlled between 118 and 140 mV under the simulated conditions [51].

Puig et al. [52] studied the effect of pH on electricity

production and contaminant dynamics using MFCs. To investigate these effects, an air-cathode MFC was used to treat urban wastewater by adjusting the pH between 6 and 10. The short-term tests showed that the highest power production ($0.66 \text{ W}\cdot\text{m}^{-3}$) was at pH 9.5. The MFC operation was in continuous control mode for 30 days and at the optimal pH improved the performance of the cell relative to power generation to $1.8 \text{ W}\cdot\text{m}^{-3}$. Organic matter removal (77% of influent COD) and physical ammonium loss were directly influenced by pH and followed the same behavior as the power generation. At a pH higher than the optimal one, anodic bacteria were affected, and power generation ceased. However, biological nitrogen processes and phosphorus dynamics were independent of the exoelectrogenic bacteria [52].

Luo et al. [53] developed a MFC cathode by immobilizing the enzyme, laccase, in polymer matrix (Nafion) on carbon paper together with carbon nanoparticles and a redox mediator (2,22 -azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt). The results from characterization of the immobilized laccase on the electrode indicated that the immobilization preserved laccase activity. Electrochemical measurements demonstrated that the Nafion film can facilitate electron exchange between the electroactive domain of laccase and the electrode. The electrocatalysis capabilities of the laccase-coated electrode were characterized by cyclic voltammogram (CV), which showed the electrode facilitated direct and surface-controlled redox reactions in a 0.1 M citrate buffer solution (pH 5.0). Additionally, when installed in a two-chamber MFC, the laccase-coated cathode demonstrated a strong catalytic potential that was comparable to a Pt-catalyzed cathode [53].

Wen et al. [54] explored a new approach to improve MFC performance and electron transfer rate through the addition of rhamnolipid. Results demonstrated that for an air-cathode MFC operating on 1 g L^{-1} glucose with different additions of rhamnolipid from 0 to 80 mg L^{-1} , the open circuit voltage (OCV) was 1.8-fold increased from 483 to 878 mV, the maximum power density was 12.5-fold increased from 22 to 275 W m^{-3} (0.6 to 7.6 W m^{-2}), while the corresponding current density of the maximum power density was 11.4-fold increased from 1.8 to 20.6 A m^{-2} , respectively. Anode discharge analysis suggested that the improvement of electron transfer from bacteria to anode can be attributed to the addition of

rhamnolipid. The high power density achieved here was due to the increase of permeability of cell membranes by the addition of rhamnolipid, which reduced the electron transfer resistance through the cell membrane and promoted the electron transfer rate and number, consequently enhancing the current and power output. A promising way of utilizing bio-surfactant to improve the energy generation of MFC was demonstrated by the authors [54].

Single and double chamber MFCs were tested in batch mode at different temperatures ranging from 4 to $35 \text{ }^\circ\text{C}$ by Guerrero et al. [55] and results were analysed in terms of efficiency in soluble organic matter removal and capability of energy generation. Brewery wastewater diluted in domestic wastewater (initial soluble chemical oxygen demand of 1200 and 492 mg L^{-1} of volatile suspended solids) was the source of carbon and inoculum for the experiments. Control reactors (sealed container with support for biofilm formation) as well as baseline reactors (sealed container with no support) were run in parallel to the MFCs at each temperature to assess the differences between water treatment including electrochemical processes and conventional anaerobic digestion (in the presence of a biofilm, or by planktonic cells). MFCs showed improvements regarding rate and extent of COD removal in comparison to control and baseline reactors at low temperatures (4, 8 and $15 \text{ }^\circ\text{C}$), whilst differences became negligible at higher temperatures (20,25,30 and $35 \text{ }^\circ\text{C}$). Temperature was a crucial factor in the yield of MFCs both for COD removal and electricity production with results that ranged from 58% final COD removal and maximum power of 15.1 mW m^{-3} reactor (8.1 mW m^{-2} cathode) during polarization at $4 \text{ }^\circ\text{C}$, to 94% final COD removal and maximum power of 174.0 mW m^{-3} reactor (92.8 mW m^{-2} cathode) at $35 \text{ }^\circ\text{C}$ for single chamber MFCs with carbon cloth-based cathodes. Bioelectrochemical processes in these MFCs were found to have a temperature coefficient, Q_{10} of 1.6. A membrane-based cathode configuration was tested and gave promising results at $4 \text{ }^\circ\text{C}$, where a maximum power output of 294.6 mW m^{-3} reactor (98.1 mW m^{-2} cathode) was obtained during polarization and a maximum Coulombic efficiency (Y_Q) of 25% was achieved. This exceeded the performance at $35 \text{ }^\circ\text{C}$ with cloth-based cathodes (174.0 mW m^{-3} ; Y_Q 1.76%) [55].

The promise of generating electricity from the oxidation of organic substances using metal-reducing

bacteria is drawing attention as an alternate form of bio-technology with positive environmental implications. Kim and Lee [56] examined various experimental factors to obtain the maximum power output in a dual-chamber mediator-less MFC using *Geobacter sulfurreducens* and acetate as an electron donor in a semi-continuous mode. The *G. sulfurreducens* culture conditions were optimized in a nutrient buffer containing 20 mM of acetate and 50 mM of fumarate at pH 6.8 and 30 °C. For use in the MFC system, electrodes were made with carbon paper (area: 11.5 cm²) and spaced 1.5 cm apart. Once the MFC was inoculated with the pre-cultured *G. sulfurreducens* in the anode chamber and while air was continuously sparged to the cathode chamber, the cells produced electricity stably over 60 days with the regular addition of 20 mM acetate, generating the maximum power density of 7 mW/m² with a 5000 &! load. The current output was significantly increased by 1.6 times after 20 days of incubation under the same experimental conditions when the carbon-paper anode was coated with carbon nanotubes [56].

An et al. [57] indicated the possibility of harvesting electricity from the surface of a tidal mud flat using a cylindrical-type sediment SMFC a marine mud battery (MMB) which can be applied in a sea environment where the ebb and flow occur due to tidal difference. In addition, the authors indirectly investigated the influence of ebb and flow in a lab using aeration, argon gassing, and by agitating the cathodic solution. The MMBs consisted of cylindrical acrylic compartments containing a nylon membrane, an anode, and a cathode in a single body. The MMBs were stuck vertically into an artificial tidal mud flat such that the anode electrode was in direct contact with the tidal mud surface. As a result, the maximum current and power density generated were 35 mA/m² and 9 mW/m², respectively, thus verifying that it is possible to harvest electricity from the surface of a tidal mud flat using an MMB without burying the anode electrode in the tidal mud. Furthermore, the results of tests using an artificial turbulence flow showed the flow induced by the tidal ebb and flow could allow the performance of MMBs to be enhanced [57].

The abiotic cathodes usually require a catalyst such as Pt to enhance power production, increasing the cost and lowering the operational sustainability. Mao et al. [58] investigated the performance of a

biocathode MFC biocatalyzed by ferro/manganese-oxidizing bacteria. A scanning electron microscopy with an energy-dispersive spectrometer (SEM-EDS) was used to characterize the cathode and analyzed the elements of cathode. The amount of ferro/manganese-oxidizing bacteria in the biocathode was examined. In batch-fed systems, the maximum open circuit voltage (OCV) was between 700 and 800 mV and the maximum cell potential difference was higher than 600 mV with an external resistance of 100 Ω. The maximum power density was 32 W m⁻³ MFC for batch-fed systems (20–40% Coulombic yield) and 28 W m⁻³ MFC for a continuous system with an acetate loading rate of 1.0 kg COD m⁻³ day⁻¹. The results of SEM-EDS clearly showed that cathode was impregnated with iron and manganese. The amount of ferro/manganese-oxidizing bacteria was $(7.5\text{--}20.0) \times 10^5$ MPN mL⁻¹ in the biocathode. This kind of biocathodes alleviates the need to use noble catalysts for the reduction of oxygen, which step forward towards large-scale application of MFCs [58].

Pinto et al. [59] evaluated the impact of the external resistance (electrical load) on the long-term performance of a MFC and demonstrated the real-time optimization of the external resistance. For this purpose, acetate-fed MFCs were operated at external resistances, which were above, below, or equal to the internal resistance of a corresponding MFC. A perturbation/observation algorithm was used for the real-time optimal selection of the external resistance. MFC operation at the optimal external resistance resulted in increased power output, improved Coulombic efficiency, and low methane production. Furthermore, the efficiency of the perturbation/observation algorithm for maximizing long-term MFC performance was confirmed by operating an MFC fed with synthetic wastewater for over 40 days. In this report an average Coulombic efficiency of 29% was achieved [59].

Membrane separators reduce oxygen flux from the cathode into the anolyte in MFCs, but water accumulation and pH gradients between the separator and cathode reduces performance. Air cathodes were spray-coated (water-facing side) with anion exchange, cation exchange and neutral polymer coatings of different thicknesses to incorporate the separator into the cathode by Watson et al. [60]. The anion exchange polymer coating resulted in greater power density (1167 ± 135 mW m⁻²) than a cation

exchange coating ($439 \pm 2 \text{ mW m}^{-2}$). This power output was similar to that produced by a Nafion-coated cathode ($1114 \pm 174 \text{ mW m}^{-2}$), and slightly lower than the uncoated cathode ($1384 \pm 82 \text{ mW m}^{-2}$). Thicker coatings reduced oxygen diffusion into the electrolyte and increased coulombic efficiency (CE = 56–64%) relative to an uncoated cathode ($29 \pm 8\%$), but decreased power production ($255\text{--}574 \text{ mW m}^{-2}$). Electrochemical characterization of the cathodes *ex situ* to the MFC showed that the cathodes with the lowest charge transfer resistance and the highest oxygen reduction activity produced the most power in MFC tests. The results on hydrophilic cathode separator layers revealed a trade off between power and CE. Cathodes coated with a thin coating of anion exchange polymer show promise for controlling oxygen transfer while minimally affecting power production [60].

The electricity production of *Shewanella*-inoculated MFCs under a magnetic field (MF) exposure was investigated by Li et al. [61]. The persistency of the MF effect and the effects of MF intensity and direction on MFC performance were also evaluated. Application of a 100-mT static MF to the MFCs improved electricity production considerably with an increase in the maximum voltage by 20–27%, depending on the reactor configuration and medium. A more conspicuous improvement by over two times in the electricity generation was observed by the authors in a three-electrode cell under an MF exposure. Such an enhancement effect was further confirmed by the variation of the MFC voltage when applying different MF strategies. It was found that the MF effect was immediate and reversible, and some adverse effect occurred when the MF was suddenly removed. The chemical, electrochemical and emission-excision matrix (EEM) spectra analyses demonstrate an enhanced bioelectrochemical activity of *Shewanella* under MF, and no significant acceleration of mediator secretion was found. The improvement in electricity production of MFCs under MF exposure was mainly attributed to the enhanced bioelectrochemical activity, possibly through the oxidative stress mechanism [61].

The MFCs have attracted increasing interests in both environmental and energy fields. Among the various MFC configurations, miniature microbial fuel cell (mini-MFC) have a great potential for the application in medical, communication and other areas because of its miniature volume and high output power density.

In this work, a 25- μL single-chamber mini-MFC was fabricated by Chen et al. [62] using the photolithography technique. The plate-shaped gold anodic electrode in the mini-MFC showed a higher electrochemical activity than the stripe-shaped one. A biofilm of *Shewanella oneidensis* MR-1 was formed on the surface of gold electrode in the micro-liter-scale MFCs. As a result, a maximum power density of 29 mW/m^2 and a maximum current density of 2148 mA/m^2 were achieved by the authors in this single-chamber mini-MFC [62].

The electrical response of photosynthetic microbial cells (PMCs) containing *Spirulina platensis* was investigated by Fu et al. [63] to assess the feasibility of using these PMCs as instant-use and portable devices. The PMCs were constructed without membranes, mediators, or any additive organic substrates. Once *S. platensis* was attached to an anode, voltage was instantly obtained and ready for application. Since no additional substrate was required as fuel for electricity generation, these PMCs were different from typical microbial fuel cells. PMCs were examined under various operating conditions, and they performed under high open circuit voltage (OCV) with high power output in the dark, short electrode spacing (2 cm), low pH value (5.5), and high temperature ($40 \text{ }^\circ\text{C}$) conditions. According to Ohm's Law and the Nernst equation, the superior OCV and power output were caused by high ionic strength, low internal resistance, and high temperature. Additionally, two equal-sized PMCs were arranged in serial and parallel configurations. The former yielded higher voltage, while the latter yielded higher current density [63].

Proton exchange membranes (PEMs) are typically used in two-chamber MFCs to separate the anode and cathode chambers while allowing protons to pass between the chambers. However, PEMs such as Nafion are not cost-effective. To reduce the cost of MFCs, Tang et al. [64] examined the performances of cellulose acetate microfiltration membranes in a two-chamber microbial fuel cell using acetate. The internal resistance, the maximum power density and the coulombic efficiency (CE) of the microfiltration membrane MFC (MMMFC) were 263Ω , $0.831 \pm 0.016 \text{ W/m}^2$ and $38.5 \pm 3.5\%$, respectively, in a fed-batch mode, while the corresponding values of the MFC using a PEM were 267Ω , $0.872 \pm 0.021 \text{ W/m}^2$ and $74.7 \pm 4.6\%$, respectively. The authors further used the MMMFC for poultry

wastewater treatment. The maximum power density of $0.746 \pm 0.024 \text{ W/m}^2$ and CE of $35.3 \pm 3.2\%$ were achieved when the poultry wastewater containing 566 mg/L COD was used, removing $81.6 \pm 6.6\%$ of the COD. These results demonstrate microfiltration membranes, compared with PEMs, have a similar internal resistance and reduce pH gradient across the membrane. The MMMFC was effective for poultry wastewater treatment with high COD removal [64].

Growing energy needs and concerns about environmental pollution have stimulated increased interest in the research and application of microbial fuel cell (MFC) systems. Hu et al. [65] investigated the possible electricity production with nitrogenous heterocyclic (N-heterocyclic) compounds degradation in the MFCs. Two-chamber MFCs were designed and inoculated with anaerobic sludge acclimated for several months. The experiments were conducted to test the potential for biodegradation of refractory organic matters and electricity generation using representative N-heterocyclic compounds such as pyridine, quinoline and indole. A maximum voltage of 524 mV , 494 mV , 413 mV (based on an external resistance of 1000Ω), and the corresponding maximum power densities of 228.8 mW m^{-2} , 203.4 mW m^{-2} , 142.1 mW m^{-2} were obtained from pyridine, quinoline, and indole, respectively. The maximum degradation efficiency of these substrates and COD (chemical oxygen demand) removal were up to 90% and 88% , respectively. The metabolic intermediate products were detected by GC/MS analyses of the anode solution. The results indicated about the use of N-heterocyclic compound as the MFC fuel in practical applications of wastewater treatment [65].

In the existing MFCs, the use of platinized electrodes and Nafion[®] as proton exchange membrane (PEM) leads to high costs leading to a burden for wastewater treatment. Pant et al. [66] reported two different novel electrode materials which can replace conventional platinized electrodes and can be used as very efficient oxygen reducing cathodes. Further, a novel membrane which can be used as an ion permeable membrane (Zirfon[®]) can replace Nafion[®] as the membrane of choice in MFCs. The above mentioned gas porous electrodes were first tested in an electrochemical half cell configuration for their ability to reduce oxygen and later in a full MFC set up. It was observed that these non-platinized air

electrodes perform very well in the presence of acetate under MFC conditions (pH 7, room temperature) for oxygen reduction. Current densities of 0.43 mA cm^{-2} for a non-platinized graphite electrode and 0.6 mA cm^{-2} for a non-platinized activated charcoal electrode at $200 \text{ mV vs. Ag/AgCl}$ of applied potential were obtained. The proposed ion permeable membrane, Zirfon[®] was tested for its oxygen mass transfer coefficient, K_0 which was compared with Nafion[®]. The K_0 for Zirfon[®] was found to be $1.9 \times 10^{-3} \text{ cm s}^{-1}$ [66].

Reduction of the compounds using MFC: Liu et al. [67] performed the *in-situ* reduction of Cr(VI) at a carbon felt cathode in an air-cathode dual-chamber MFC. The reduction of Cr(VI) was found to be strongly associated with the electrogenerated H_2O_2 at the cathode driven by iron-reducing bacteria. At pH 2.0, only 42.5% of Cr(VI) was reduced after 12 h in the nitrogen-bubbling-cathode MFC, while complete reduction of Cr(VI) was achieved in 4 h in the air-bubbling-cathode MFC in which the reduction of oxygen to H_2O_2 was confirmed. Conditions that affected the efficiency of the reduction of Cr(VI) were evaluated experimentally, including the cathodic electrolyte pH, the type of iron-reducing species and the addition of redox mediators. The results showed that the efficient reduction of Cr(VI) could be achieved with an air-bubbling-cathode MFC [67].

Polyaniline/carbon black (PANI/C) composite-supported iron phthalocyanine (FePc) (PANI/C/FePc) was investigated as a catalyst by Yuan et al. [68] for the oxygen reduction reaction (ORR) in an air-cathode MFC. The electrocatalytic activity of the PANI/C/FePc towards the ORR was evaluated using cyclic voltammogram and linear scan voltammogram methods. In comparison with that of carbon-supported FePc electrode, the peak potential of the ORR at the PANI/C/FePc electrode shifted toward positive potential, and the peak current was greatly increased, suggesting the enhanced activity of FePc absorbed onto PANI/C. Additionally, the results of the MFC experiments showed that PANI/C/FePc is well suitable to be the cathode material for MFCs. The maximum power density of 630.5 mW m^{-2} with the PANI/C/FePc cathode was higher than that of 336.6 mW m^{-2} with the C/FePc cathode, and even higher than that of 575.6 mW m^{-2} with a Pt cathode [68].

Wu et al. [69] prepared reduced graphene sheets

(RGSs) via chemical reduction of graphite oxide and their morphology was characterized by atomic force microscopy. The electrochemical reduction of oxygen (O_2) with RGSs was studied by cyclic, rotating disk electrode, and rotating ring-disk electrode voltammetry using the RGSs-modified glassy carbon (RGSs/GC) electrode in 3.5% NaCl solution. The results showed the involvement of three steps in O_2 reduction at the RGSs/GC electrode: electrochemical reduction of O_2 to H_2O_2 mediated by quinone-like groups on the RGSs surface, a direct 2-electron reduction of O_2 , and reduction of the H_2O_2 produced to H_2O . The modification of RGSs results in an obvious positive shift of the peak potential and a larger current density. The kinetics study shows that the number of electrons transferred for O_2 reduction can reach to 3.0 at potentials of the first reduction step, indicating RGSs can effectively catalyze the disproportionation of H_2O_2 . Such catalytic activity of RGSs enables a 4-electron reduction of O_2 at a relatively low overpotential in neutral media. The authors explored RGSs as a potential electrode material for microbial fuel cells in this work [69].

In a study, Fu et al. [70] focused on the preparation of a new type of Prussian Blue/polyaniline (PB/PANI)-modified electrode as oxygen reduction cathode, and its availability in MFC for biological power generation. The PB/PANI-modified electrode was prepared by electrochemical and chemical methods and both type exhibited good electrocatalytic reactivity for oxygen reduction in acidic electrolyte. The MFC with PB/PANI-modified cathode aerated by either oxygen or air was shown to yield a maximum power density being the same with that of the MFC with liquid-state ferricyanide cathode, and exhibited an excellent duration as indicated by stable cathode potential for more than eight operating circles. This study suggests a promising potential to utilize this novel electrode as an effective alternative to platinum for oxygen reduction in MFC system without losing sustainability [70].

You et al. [71] reported the fabrication of a new membrane electrode assembly by using stainless steel mesh (SSM) as raw material and its effectiveness as gas diffusion electrode (GDE) for electrochemical oxygen reduction in MFC. Based on feeding glucose (0.5 g L^{-1}) substrate to a single-chambered MFC, power generation using SSM-based GDE was increased with the decrease of polytetrafluoroethylene (PTFE) content applied during fabrication,

reaching the optimum power density of 951.6 mW m^{-2} at 20% PTFE. Repeatable cell voltage of 0.51 V (external resistance of 400Ω) and maximum power density of 951.6 mW m^{-2} produced for the MFC with SSM-based GDE are comparable to that of 0.52 V and 972.6 mW m^{-2} , respectively obtained for the MFC containing typical carbon cloth (CC)-made GDE. Besides, Coulombic efficiency (CE) was found higher for GDE (SSM or CC) with membrane assembly than without, which results preliminarily from the mitigation of Coulombic loss being associated with oxygen diffusion and substrate crossover. This study demonstrates that with its good electrical conductivity and much lower cost, the SSM-made GDE renders a promising alternative for efficient and more economically viable material to conventional typical carbon for power production from biomass in MFC [71].

Nitrification to nitrite (nitritation process) followed by reduction to dinitrogen gas decreases the energy demand and the carbon requirements of the overall process for nitrogen removal. Puig et al. [72] studied autotrophic nitrite removal in the cathode of MFCs. Special attention was paid to determine whether nitrite is used as the electron acceptor by exoelectrogenic bacteria (biologic reaction) or by graphite electrodes (abiotic reaction). The results demonstrated that after a nitrate pulse at the cathode, nitrite was initially accumulated; subsequently, nitrite was removed. Nitrite and nitrate can be used interchangeably as an electron acceptor by exoelectrogenic bacteria for nitrogen reduction from wastewater while producing bioelectricity. However, if oxygen is present in the cathode chamber, nitrite is oxidised via biological or electrochemical processes. The identification of a dominant bacterial member similar to *Oligotropha carboxidovorans* confirms that autotrophic denitrification is the main metabolism mechanism in the cathode of a MFC [72].

Biofilms formed in aerobic seawater on stainless steel are known to be efficient catalysts for the electrochemical reduction of oxygen. Parot et al. [73] selected seven bacterial isolates based on their genomic analysis and a cyclic voltammetry (CV) procedure was implemented to check their electrocatalytic activity towards oxygen reduction. All isolates exhibited close catalytic characteristics. Comparison between CVs recorded with glassy carbon and pyrolytic graphite electrodes showed that the catalytic effect was not correlated with the surface

area covered by the cells. The low catalytic effect obtained with filtered isolates indicated the involvement of released redox compounds, which was confirmed by CVs performed with adsorbed iron-porphyrin. None of the isolates were able to form electro-active biofilms under constant polarization. The capacity to catalyze oxygen reduction is shown to be a widespread property among bacteria, but the property detected by CV not conferred the ability to achieve stable oxygen reduction under constant polarization [73].

Kim et al. [74] prepared Cobalt-naphthalocyanine (CoNPc) by heat treatment for cathode catalysts to be used in MFCs. Four different catalysts (Carbon black, NPc/C, CoNPc/C, Pt/C) were compared and characterized using XPS, EDAX and TEM. The electrochemical characteristics of oxygen reduction reaction (ORR) were compared by cyclic voltammetry (CV) and linear sweep voltammetry (LSV). The Co-macrocylic complex improved the catalyst dispersion and oxygen reduction reaction of CoNPc/C. The maximum power of CoNPc/C was 64.7 mW/m² at 0.25 mA as compared with 81.3 mW/m² of Pt/C, 29.7 mW/m² of NPc/C and 9.3 mW/m² of carbon black when the cathodes were implemented in H-type MFCs. The steady state cell, cathode and anode potential of MFC with using CoNPc/C were comparable to those of Pt/C [74].

Birry et al. [75] made catalysts for the oxygen reduction reaction (ORR) in a MFC by the impregnation on carbon black of Fe^{II} acetate (FeAc), Cl-Fe^{III} tetramethoxyphenyl porphyrin (ClFeTMPP), and Fe^{II} phthalocyanine (FePc). These materials were subsequently pyrolyzed at a high temperature. The ORR activity of all Fe-based catalysts was measured at pH 7 with a rotating disk electrode (RDE) and their performance for electricity production was then verified in a continuous flow MFC. Catalysts prepared with FeAc and pyrolyzed in NH₃ showed poor activity in RDE tests as well as a poor performance in a MFC. The ORR activity and fuel cell performance for catalysts prepared with ClFeTMPP and FePc and pyrolyzed in Ar were significantly higher and comparable for both precursors. The iron loading was optimized for FePc-based catalysts. With a constant catalyst load of 2 mg cm⁻² in a MFC, the highest power output (550–590 mW/m²) was observed when the Fe content was 0.5–0.8 wt%, corresponding to only 0.01–0.16 mg Fe/cm². A similar power output was observed using a Pt-based carbon cloth cathode

containing 0.5 mg Pt/cm². Long-term stability of the Fe-based cathode (0.5 wt% Fe) was confirmed over 20 days of MFC testing [75].

Degradation of wastes and toxic chemicals using MFC technology: Glycerol degradation with electricity production by a pure culture of *Bacillus subtilis* in a single-chamber air cathode of MFC was demonstrated by Nimje et al. [76]. Steady state polarization curves indicated a maximum power density of 0.06 mW/cm² with an optimal external resistance of 390 Ω. Analysis of the effect of pH on MFC performance demonstrated that electricity generation was sustained over a long period of time under neutral to alkaline conditions. Cyclic voltammetry exhibited the increasing electrochemical activity with the increase of pH of 7, 8 and 9. Voltammetric studies also demonstrated about a two-electron transfer mechanism occurrence in the reactor. The achieved low Coulombic efficiency of 23.08% was attributed to the loss of electrons for various activities other than electricity generation. This study describes the application of glycerol in MFC and this can contribute to transformation of the biodiesel industry to a more environmentally friendly microbial fuel cell-based technology [76].

Li and Ni [77] used a two-chamber MFC to treat *Dioscorea zingiberensis* processing wastewater and generate electricity. The contaminant degradation process was systematically investigated with the help of UV-Vis, FTIR spectra and GC-MS. The results showed that the COD removal efficiency of the MFC reached 93.5% and the maximum power density achieved 175 mW/m². In the anodic chamber, low molecule weight acid, sugars and cellulose in *D. zingiberensis* processing wastewater were completely consumed, while complicated contaminants including some furanic and phenolic compounds were decomposed under co-metabolism process. In the cathodic chamber, fatty ester and alkene generated in the anodic chamber were removed, and aromatic compounds were further degraded. Aromatic ester and N-containing compounds were detected as the main residual contaminants by GC-MS. Compared to the effluents of anaerobic digestion and biological aerated filter, fewer and simpler aromatic pollutants existed in the effluents of MFC [77].

Bio (microbial) fuel cell with *Saccharomyces cerevisiae* as anodic biocatalyst was evaluated by Raghavulu et al. [78] in terms of power generation

and substrate degradation at three redox conditions (5.0, 6.0 and 7.0) and fuel cell was operated in single chamber (open-air cathode) configuration without mediators using non-catalyzed graphite as electrodes. The performance was further studied with increasing loading rate (OLRI, 0.91 kg COD/m³-day; OLRII, 1.43 kg COD/m³). Higher current density was observed at pH 6.0 [160.36 mA/m² (OLRI); 282.83 mA/m² (OLRII)] than pH 5.0 (137.24 mA/m²) and pH 7.0 (129.25 mA/m²). Bio-electrochemical behavior of fuel cell was evaluated using cyclic voltammetry which showed the presence of redox mediators (NADH/NAD⁺; FADH/FAD⁺). Higher electron discharge was observed at pH 6.0, suggesting higher proton shuttling through the involvement of different redox mediators. The results indicated that this study can be extended to treat high strength wastewaters with simultaneous power generation [78].

Katuri et al. [79] investigated the influence of external load on the composition of the anodic biofilm microbial community and biomass yield in a microbial fuel cell fed with glucose and domestic wastewater was used as source of electrogens. Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S rRNA gene fragments revealed distinct differences in anodic bacterial communities formed at the anode of each MFC operated under a different external load. These results implied that in a MFC, electrogenic bacteria were enriched under higher current densities, i.e., low external load, and were able to sustain better current and effluent quality. The influence of the external resistance applied to the MFCs during formation of the bacterial communities from sewage wastewater was shown to have no significant effect on power performance of the MFCs nor to have a significant influence on their anodic activity with both glucose and brewery wastewater as fuel. As expected, current generation, COD removal and the biomass yield were all directly influenced by the external load. Significantly, when operated under lower external load, the biomass yield in the MFC was less than that of in conventional anaerobic digestion. [79].

Puig et al. [80] assessed the feasibility of using MFCs in landfill leachate treatment and electricity production under high levels of nitrogen concentration (6033 mg N L⁻¹) and conductivity (73,588 μ S cm⁻¹). An air-cathode MFC was used over a period of 155 days to treat urban landfill leachate. Up to

8.5 kg COD m⁻³ d⁻¹ of biodegradable organic matter was removed at the same time electricity (344 mW m⁻³) was also produced. Nitrogen compounds suffered transformations in the MFC. Ammonium ions were oxidized to nitrite using oxygen diffused from the membrane. However, at high free ammonia concentrations (around 900 mg N-NH₃ L⁻¹), the activity of nitrifier microorganisms was inhibited. Ammonium reduction was also resulted from ammonium transfer through the membrane or from ammonia loss. High salinity content benefited the MFC performance by increasing power production and decreasing the internal resistance [80].

Practical applications of MFCs for wastewater treatment will require operation of these systems over a wide range of wastewater temperatures. MFCs at room or higher temperatures (20–35 °C) are relatively well studied compared those at lower temperatures. A MFC performance was examined by Cheng et al. [81] over a temperature range of 4–30 °C in terms of startup time needed for reproducible power cycles and performance. MFCs initially operated at 15 °C or higher all attained a reproducible cycles of power generation, but the startup time to reach stable operation increased from 50 h at 30 °C to 210 h at 15 °C. At temperatures below 15 °C, MFCs did not produce appreciable power even after one month of operation. If a MFC was first started up at temperature of 30 °C, then reproducible cycles of power generation could be achieved at even the two lowest temperatures of 4 °C and 10 °C. Power production increased linearly with temperature at a rate of 33 ± 4 mW °C⁻¹ from 425 ± 2 mW m⁻² at 4 °C to 1260 ± 10 mW m⁻² at 30 °C. Coulombic efficiency decreased by 45% over this same temperature range, or from CE = 31% at 4 °C to CE = 17% at 30 °C. Results demonstrated that MFCs can effectively be operated over a wide range of temperatures, but the authors findings have important implications for the startup of larger scale reactors where low wastewater temperatures could delay or prevent adequate startup of the system [81].

MFC can be an efficient sludge treatment unit in regard of rates and extents of total chemical oxygen demand (TCOD) removal, particularly when ultrasound was applied to pretreat the sludge. In a study, Jiang et. al. [82] characterized the organic matter in sludge before and after MFC treatment with or without ultrasound as a pretreatment stage. The 5-d MFC tests with electric load significantly

enhanced TCOD removal rate from 11.3% to 19.2% for raw sludge and from 25% to 57% for sludge pretreated with $>0.6 \text{ W ml}^{-1}$ ultrasound, using conventional anaerobic digestion test (without electric load) as control. The aromatic proteins, soluble microbial byproduct-like fluorescent compounds and carboxylic components, aliphatic components (C–H related), hydrocarbon and carbohydrate materials were identified to be principally released by ultrasound pretreatment and the fuels in the study [82].

Xie et al. [83] implemented a coupled MFC system comprising of an oxic-biocathode MFC (O-MFC) and an anoxic-biocathode MFC (A-MFC) for simultaneous removal of carbon and nitrogen from a synthetic wastewater. The chemical oxygen demand (COD) of the influent was mainly reduced at the anodes of the two MFCs; ammonium was oxidized to nitrate in the O-MFC's cathode, and nitrate was electrochemically denitrified in the A-MFC's cathode. The coupled MFC system reached power densities of 14 W/m^3 net cathodic compartment (NCC) and 7.2 W/m^3 NCC for the O-MFC and the A-MFC, respectively. In addition, the MFC system obtained a maximum COD, $\text{NH}_4^+\text{-N}$ and TN removal rate of 98.8%, 97.4% and 97.3%, respectively, at an A-MFC external resistance of 5Ω , a recirculation ratio (recirculated flow to total influent flow) of 2:1, and an influent flow ratio (O-MFC anode flow to A-MFC anode flow) of 1:1 [83].

To understand how cathode performance and substrates affected communities that evolved in these reactors over long periods of time, a study was conducted by Kiely et al [84], here MFCs were operated for more than 1 year with individual end products of lignocellulose fermentation (acetic acid, formic acid, lactic acid, succinic acid, or ethanol). Large variations in reactor performance were primarily due to the specific substrates, with power densities ranging from 835 ± 21 to $62 \pm 1 \text{ mW/m}^3$. Cathodes performance degraded over time, as shown by an increase in power of up to 26% when the cathode biofilm was removed, and 118% using new cathodes. Communities that developed on the anodes included exoelectrogenic families, such as *Rhodobacteraceae*, *Geobacteraceae*, and *Peptococcaceae*, with the *Deltaproteobacteria* dominating most reactors. *Pelobacter propionicus* was the predominant member in reactors fed acetic acid, and it was abundant in several other MFCs. These results

provide valuable insights into the effects of long-term MFC operation on reactor performance [84].

Nitrogen removal mainly relies on sequential nitrification and denitrification in wastewater treatment. MFCs are innovative wastewater treatment techniques for pollution control and energy generation. Yu et al. [85] constructed bench-scale wastewater treatment systems using membrane-aerated MFC (MAMFC) and diffuser-aerated MFC (DAMFC) techniques for simultaneous removal of carbonaceous and nitrogenous pollutants and electricity production from wastewater. During 210 days of continuous flow operation, when the dissolved oxygen (DO) in the cathodic compartment was kept at 2 mg/L , both reactors demonstrated high COD removal ($>99\%$) and high ammonia removal ($>99\%$) but low nitrogen removal ($<20\%$). When a lower DO (0.5 mg/L) was maintained after day 121, both the MFC-based reactors still showed excellent COD removal ($>97\%$). However, the nitrogen removal of MAMFC (52%) was 2-fold higher than that of DAMFC (24%), indicating an enhanced performance of denitrification after DO reduction in the cathodic compartment of the MAMFC. Meanwhile, terminal restriction fragment length polymorphism (T-RFLP) analysis of ammonia-oxidizing bacteria (AOB) population in the MAMFC indicated the diversity of AOB with equally important *Nitrosospira* and *Nitrosomonas* species present in the cathodic biofilm after DO reduction. The average voltage output in the MAMFC was significantly higher than that in DAMFC under both DO conditions. The results suggest that MAMFC systems have the potential for wastewater treatment with improved nitrogen removal and electricity production [85].

Luo et al. [86] combined the MFC with the Fenton-like technology to simultaneously generate electricity and degrade refractory contaminants in both anode and cathode chambers. The maximum power density achieved was 15.9 W/m^3 at an initial pH of 3.0 in the MFC. In the anode chamber, approximately 100% of furfural and 96% COD were removed at the end of a cycle. In the cathode chamber, the Fenton-like reaction with FeVO_4 as a catalyst enhanced the removal of AO7 and COD. The removal rates of AO7 and COD reached 89% and 81%, respectively. The optimal pH value and FeVO_4 dosage toward degrading AO7 were about 3.0 and 0.8 g, respectively. Furthermore, a two-way catalyst mechanism of FeVO_4 and the contaminant degradation pathway in

the MFC were explored [86].

Detection of toxic compounds using MFC:

MFCs have been used for several years as biosensors for measuring environmental parameters such as biochemical oxygen demand and water toxicity. Davila et al. [87] reported the detection of toxic matter using a novel silicon-based MFC. Like other existing toxicity sensors based on MFCs, this device is capable of detecting the variation on the current produced by the cell when toxic compounds are present in the medium. The MFC approach presented in this work was aimed to obtain a simple, compact and planar device for its further application as a biosensor in the design and fabrication of equipment for toxicity monitoring. It consists of a proton exchange membrane placed between two microfabricated silicon plates that act as current collectors. An array of square $80\ \mu\text{m} \times 80\ \mu\text{m}$ vertical channels, $300\ \mu\text{m}$ deep, have been defined through the plates over an area of $6\ \text{mm} \times 6\ \text{mm}$. The final testing assembly incorporates two perspex pieces positioned onto the plates as reservoirs with a working volume of $144\ \mu\text{L}$ per compartment. The operation of the microdevice as a direct electron transfer MFC has been validated by comparing its performance against a larger scale MFC, run under the same conditions. The device has been tested as a toxicity sensor by setting it at a fixed current while monitoring changes in the output power. A drop in the power production is observed when a toxic compound is added to the anode compartment. The compact design of the device made it suitable for its incorporation into measurement equipment either as an individual device or as an array of sensors for high throughput processing [87].

Parameswaran, et al. [88] incorporated homo-acetogens in the anode of a microbial electrolysis cell (MEC) fed with H_2 as sole electron donor allowed current densities similar to acetate-fed biofilm anodes ($\sim 10\ \text{A}/\text{m}^2$). Evidence for homo-acetogens including accumulation of acetate at high concentrations (up to $18\ \text{mM}$) in the anode compartment and detection of formate, a known intermediate during reductive acetogenesis by the acetyl-CoA pathway; and detection of formyl tetrahydrofolate synthetase (FTHFS) genes by quantitative real-time PCR are reported. Current production and acetate accumulation increased in parallel in batch and continuous mode, while both values decreased simultaneously at short hydraulic retention times (1 h) in the anode

compartment, which limited suspended homo-acetogens. Acetate produced by homo-acetogens accounted for about 88% of the current density of $10\ \text{A}/\text{m}^2$, but the current density was sustained at $4\ \text{A}/\text{m}^2$ at short hydraulic retention time because of a robust partnership of homo-acetogens and anode respiring bacteria (ARB) in the biofilm anode [88].

CONCLUSION

Biological fuel cells fall into two categories, namely microbial fuel cells and enzymatic fuel cells. The main challenge is in the coupling of the biological component of the system with the electrodes of the fuel cells. By tapping the complete multi-enzyme metabolic pathways inside living cells, microbial fuel cells could last long and could utilize complex biofuels. In this review the recently published papers and reviews on MFCs are discussed in terms of their performance, evaluation, electrode materials and applications in the area of detection, degradation, green electricity generation and environmental treatment. We believe that this contribution is able to provide benefit to the biofuel cell researchers and the beginners. Moreover, efforts are going on in our establishment to make biofuel cells to utilize in the sensing of biological agents.

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