

2.1 THIRD GENERATION BIOFUELS

Biofuels hold tremendous promise in providing energy security for the future. Biofuels are renewable, environment friendly, usable in existing engines, blendable with diesel, and available in liquid, gas, & solid form. Biofuels have been explored extensively during the last few decades [Chowdhury and Loganathan, 2019]. Based on the original raw material for biofuel production, biofuels are categorized as the first generation, second generation, and third generation. The first generation (1G) biofuels involves the use of food-based biomass feedstock like sugarcane, potato, corn, beet, sunflower, rapeseed etc. The use of 1G biofuels triggers food v/s fuel debate and is often limited by the availability of agricultural land. The direct use of food crops is highly unsustainable, particularly in highly populated developing countries. The 2G biofuels are derived from inedible portions of the plant and non-food items such as lingo-cellulosic wastes, waste cooking oil, carbon rich industry waste, etc. [Chowdhury and Loganathan, 2019]. The production of 2G biofuels is limited by the need to pretreat biomass, remove inhibitors, develop an enzymatic cocktail for hydrolysis, and develop an efficient fermenting strain.

The bottlenecks associated with the 1G and 2G biofuels switched researcher's focus towards the evolution of 3G biofuels [Brennan and Owende, 2010]. The third generation biofuels are obtained from microalgae biomass. This generation of biofuels circumvents some of the problems associated with 1G and 2G biofuels and is relatively sustainable [Nigam and Singh, 2011]. Algae is a source of several other high-value low-volume products that enable their use in a bio refinery [Chisti, 2007]. The 3G biofuels hold several advantages over 1G and 2G biofuels, such as shorter harvesting cycle, higher growth rate, and higher oil production rate [Schenk et al., 2008]. Algae cultivation does not depend on agricultural land eliminating food v/s fuel issue [S. A. Scott et al., 2010]. Estimates show that the bio-oil productivity of 10000 L/hectare/year of bio-oil can be obtained from microalgae [Alam et al., 2015].

Algae are classified into two major categories based on their external morphology, i.e. microalgae and macroalgae. Brown and red algae along with green seaweed are prominent examples of macroalgae whereas microalgae include *Chlorella*, *Spirulina* and other green algae [Demirbas, 2010]. The microalgae are superior to macroalgae in terms of oil content, microscopic cell size, and higher growth rate. Algae biomass can be converted to bioethanol, biodiesel, biomethane, biohydrogen, biochar and some value added pigments and value added products [Kumar et al., 2020]. A brief description of major third generation biofuel is presented below:-

2.1.1 Biodiesel

The biodiesel is mainly derived from the intracellular lipid of the oleaginous microalgae. The algal lipid consists of triglycerides (TAG) along with mono and diglycerides and free fatty acids. Stearic acid, palmitic acid, and oleic acid are the predominant fatty acids types in the algal lipid [Tripathi et al., 2015]. Algae biomass displays a variable amount of lipid content depending on the strain type and cultivation condition. For example, the lipid content of *Chlorella vulgaris* varies from 11% to 43% [Mitra et al., 2012]. Enamala et al., (2018), reviewed several studies and found that the lipid content varies from 2.4% to 62% of dry algal cell weight [Enamala et al., 2018].

The algal lipids convert to biodiesel through catalytic transesterification reaction between triacylglycerols and methanol. The transesterification process results in fatty acid methyl ester (FAME) and glycerol [Kumar et al., 2020]. The purified FAMEs are known as biodiesel. The lipid composition, such as the percentage of saturated fatty acids, affects the fuel properties. The algal oil contains higher unsaturated fatty acids than saturated ones, which improves cold flow and make it a suitable feedstock [Demirbaş, 2009; Tripathi et al., 2015]. However, it also triggers the production of hydroperoxide and insoluble substances which collectively lead to choking of the filter [Kumar and Thakur, 2018].

2.1.2 Biomethane

Algae biomass or leftover algal biomass after lipid extraction (Lipid extracted algae) produces biogas when subjected to anaerobic digestion. This biogas is composed of CH₄ (50-70%) and CO₂ (30-50%) [Kumar et al., 2020]. The algal biomass can generate 0.024 –0.6 L CH₄/g VS (volatile solid) or 0.2 –0.4 m³ CH₄/kg biomass. The biogas yields vary from one species to another and depend on process conditions [Milledge et al., 2019; Rabii et al., 2019]. The factors affecting biogas production include algae cell wall composition, process temperature, C/N ratio, biomass loading rate, reactor configuration, etc. [Barbot et al., 2016; McKennedy and Sherlock, 2015; Sialve et al., 2009]. The biogas production process when integrated with other bioenergy process adds value and makes it sustainable [Cesaro and Belgiorno, 2015].

2.1.3 Biochar

Biochar is produced through hydrothermal carbonization (HTC) of dry biomass [Gollakota et al., 2018]. Algae based biochar has high cation exchange capacity, lesser carbon proportion, and lesser surface area than lignocellulosic biomass based biochar [Michalak et al., 2019]. The high ash content blocks micro pores resulting in low active surface area [Leng et al., 2021]. Algae biochar also possesses several functional groups making it suitable for the remediation of inorganic and organic contaminants from wastewater [Kumar et al., 2020].

Algae based biochar has higher yield compared to other feedstocks and the yield ranges from 8.1% to 64.2% of dry biomass [Michalak et al., 2019; Yu et al., 2017].

2.1.4 Bioethanol

Algal biomass can ferment to bioethanol under anaerobic conditions. The process is mediated by yeast, bacteria and/or fungi [Minh and Hanh, 2012; Robak and Balcerek, 2018]. The algae biomass contains several polymers like mannitol and agar [Kostas et al., 2016; Offei et al., 2018]. The brown algae is rich in carbohydrates such as alginate, mannitol, laminarin, glucose, fucoidan, and cellulose [Ale and Meyer, 2013]. Similarly, the red algae has a diverse range of hydrolysable polymers, which can be converted to ethanol [Behera et al., 2015].

2.1.5 Other value-added products

Intact algae biomass or algae products find applications in industries such as food, pharmaceutical, healthcare, cosmetics etc. Algal species such as *Spirulina* and *Chlorella* serves as a food supplement and source of protein [Kumar et al., 2020]. Algal produces pigments like carotenoids, phycocyanin and chlorophyll [Barkia et al., 2019]. Carotenoids such as zeaxanthin, α -carotene, β -carotene, and lutein are antioxidants and have anticancer properties [Dickinson et al., 2017; Matos, 2017]. The polyunsaturated fatty acids (PUFA) derived from algae serves as food supplements [Lee, 2013]. In addition to this, some unconventional value-added products such as ubiquinone coenzyme Q₁₀, ubiquinol, cannabinoids, anandamids, hoshinolactum, dolastatins, endotoxins, bio-dols and several therapeutic substances are reported to obtained from algae [Abu-ghosh et al., 2021; Hans et al., 2021; Mondal et al., 2020].

2.2 OLEAGINOUS ALGAE

Algae is classified in nine groups, namely cyanobacteria (Cyanophyceae), diatoms (Bacillariophyceae), brown algae (Phaeophyceae), yellow-green algae (Xanthophyceae), red algae (Rhodophyceae), green algae (Chlorophyceae), golden algae (Chrysophyceae), “picoplankton” (Prasinophyceae and Eustigmatophyceae) and dinoflagellates (Dinophyceae) [Neto et al., 2019]. Microalgae such as *Chlorella*, *Spirulina*, *Haematococcus* and *Dunaliella* are grown commercially with a production level of several 100 tons annually [Neto et al., 2019]. These algae are a rich source of protein, carbohydrate, lipid, and other value-added products. Table 2.1 shows the list of commercially cultivated algal species and their bimolecular content.

Table 2.1: List of prominent algal species and their major cell constituents. Source: [Um and Kim, 2009]

Microalgal species	Protein	Carbohydrate	Lipid
<i>Tetraselmis maculata</i>	52	15	3
<i>Synechococcus sp.</i>	63	15	11
<i>Spirulina platensis</i>	46-63	8-14	4-9
<i>Chlorella pyrenoidosa</i>	57	26	2
<i>Chlorella vulgaris</i>	51-58	12-17	14-22
<i>Chlamydomonas reinhardtii</i>	48	17	21
<i>Anabaena cylindrica</i>	43-56	25-30	4-7
<i>Dunaliella bioculata</i>	49	4	8
<i>Porphyridium cruentum</i>	28-39	40-57	9-14
<i>Prymnesium parvum</i>	28-45	25-33	22-39
<i>Scenedesmus dimorphus</i>	8-18	21-52	16-40
<i>Scenedesmus obliquus</i>	50-56	10-17	12-14
<i>Spirogyra sp.</i>	6-20	33-64	11-21
<i>Spirulina maxima</i>	60-71	13-16	6-7

2.3 MODES OF ALGAE CULTIVATION

2.3.1 Open cultivation systems

A. Open unagitated pond

The unstirred and shallow open ponds require little effort for algae cultivation on a large scale. Natural water bodies having 50 cm depth are ideal for this kind of cultivation. The disadvantages associated with such systems include frequent contamination, slower diffusion of nutrients, and the formation of algal bloom [Chew et al., 2018].

B. Circular ponds

Circular ponds are similar to that of unstirred open ponds except that they are equipped with a stirring unit. The mixing in circular ponds is enabled by a rotating shaft which moves in axial direction in order to create a homogenous mixing of nutrients [Ting et al., 2017]. The circular ponds are also prone to contamination.

C. Raceway ponds

The raceway ponds are extensively used for the commercial production of algae biomass. Raceway ponds have a race track type design and can have a single channel or multiple channels [Ting et al., 2017]. Paddle wheels in these systems ensure mixing and homogenous suspension of algae cells.

2.3.2 Closed cultivation systems

A. Horizontal tube photo-bioreactor

The horizontal tube photo-bioreactor (PBR) has long horizontal tubes arranged as panels, walls or helices [Chew et al., 2018]. Mixing is achieved through a centrifugal pump [Klinthong et al., 2015]. The reactors can run using either natural or artificial light (Figure 2.1 A). The limitation is the requirement of large surface area.

B. Vertical tube PBRs

Vertical tube PBRs, such as airlift & bubble column PBRs has an air sparger at the bottom of the reactor enabling mixing, nutrient, and gas exchange. The liquid flow in a bubble column reactor is triggered by the air bubbles produced at the bottom of the vessel. The high surface area to volume ratio of bubbles allows efficient gas exchange. The airlift reactor contains two interconnected regions, namely, dark and illuminated zones. Air bubbles lift the liquid from dark to light zones leading to homogenous mixing of nutrients and fluids between the two zones (Figure 2.1B). Vertical tube type PBRs offers homogenous mixing, low shear stress on cells, high photosynthetic efficiency, and high algal productivities [Chew et al., 2018].

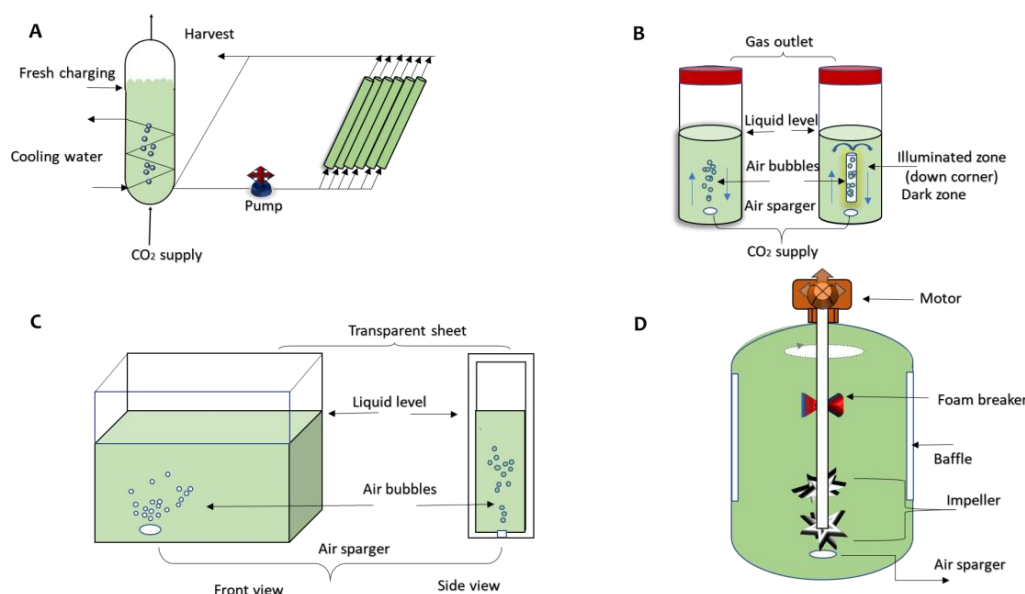


Figure 2.1: Various close cultivation systems: A) Horizontal tube PBR, B) Vertical tube PBR, C) Flat panel PBR and D) CSTR type PBR

C. Flat panel PBR

Flat panel PBRs consists of two transparent plates arranged as rectangular box (Figure 2.1 C). The light source orientation ensures equal light intensity at all positions of the reactor. The air sparger and pump enables mixing and gas exchange [Klinthong et al., 2015]. These systems have high surface area to volume ratio, suitable design for scaling up, and low level of oxygen retention inside the reactor [Ting et al., 2017].

D. Continuous stirred tank PBR

Continuous stirred tank reactor (CSTR) is similar to conventional CSTR bioreactors except for the presence of external light source in them (Figure 2.1 D). These systems offer lower productivities due to inefficient light penetration and low surface area to volume ratio [Chew et al., 2018].

2.4 BOTTLENECKS ASSOCIATED WITH EXISTING ALGAE CULTIVATION SYSTEMS

The main focus of all the commercial industries dealing with third generation biofuels is to optimize and develop efficient & cost effective approaches for maximizing the algal biomass production. However, the existing algae cultivation strategies have several drawbacks which need to be addressed in order to commercialize the third generation biofuels. The few of them are listed in table 2.2:

Table 2.2: Pros & cons associated with conventional algae cultivation systems.

Cultivation system		Pros	Cons
Open	Open ponds	<ul style="list-style-type: none"> • Easy to build handle • Ideal for mass production at relatively affordable price 	<ul style="list-style-type: none"> • Evaporation losses & prone to contamination • Requirement of large area & CO₂ transportation from source to cultivation site
Close	Tubular	<ul style="list-style-type: none"> • Ideal for outdoor cultivation • Temperature can be controlled • High biomass production 	<ul style="list-style-type: none"> • Requirement of O₂ quenching due to high DO concentration • Shading effect • Not suitable for scale-up processes
	Flat panel	<ul style="list-style-type: none"> • High algal growth rate • Comparatively lower O₂ storage • High amount of light per unit area 	<ul style="list-style-type: none"> • Difficulty in controlling temperature • Complexity in scale-up • Shading effect
	Continuous stir tank	<ul style="list-style-type: none"> • Better biomass yield due to good mixing • Minimum shading effect 	<ul style="list-style-type: none"> • High cost associated with scale-up processes • Requirement of O₂ quenching

2.5 INTEGRATING ALGAE CULTIVATION WITH BIO-ELECTROCHEMICAL SYSTEMS

Bio-electrochemical systems such as Microbial Fuel Cell (MFC) can be used for algae cultivation and bioelectricity production. MFCs finds applications in wastewater treatment, CO₂ sequestration, heavy metals removal, bio-remediation, etc. [Zhang et al., 2011]. A typical MFC consists of an anode & cathode placed in two chambers separated by an ion-exchange membrane. Oxidation of electron donor by microbial catalysts at anodic chamber gives electrons (reducing anode), CO₂, and electrical energy [Chen et al., 2019]. The electrons flow through the external circuit to be captured by the terminal electron acceptor present at the cathode [Trapero et al., 2017]. The anode and cathode chamber have differences in redox potential, which is often maintained with the help of the ion-exchange membrane. The detailed description of MFCs can be found in below sections. The electron acceptor at the cathode can be oxygen/NO₃⁻/NO₂⁻/metal ions. However, the need for a continuous supply of terminal electron acceptor in the cathode can be eliminated by growing algae in the cathodic chamber, as it produces enough oxygen through the action of photosynthesis. On the other hand, the conventional algae cultivation systems require periodic oxygen quenching, which involves further energy consumption and adds cost to the process. Therefore, the integration of algae cultivation and MFC technology can eliminate the need of oxygen quenching and external aeration in the MFC system and make the overall process sustainable and net energy producer. In addition to this, the CO₂ gas produced in the anodic chamber can promote the algal growth in the cathodic chamber. Hence, the energy and cost invested for CO₂ transportation in an open pond system can be saved (Figure 2.2).

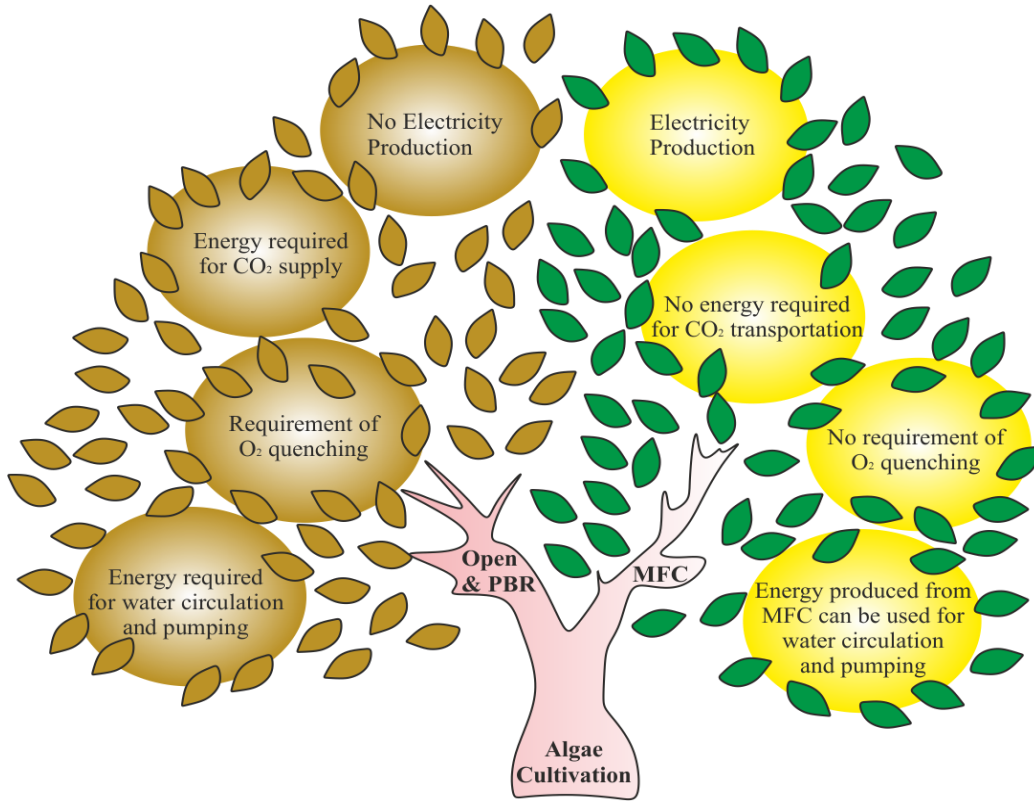
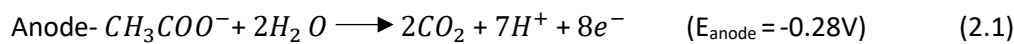


Figure 2.2: Illustration showing the comparison between conventional algae cultivation systems and MFC based algae cultivation.

2.6 MFC PRINCIPLE AND ITS COMPONENTS

The electrigenes reduce the anode by oxidizing the organic matter present in the anodic chamber. The process of anode reduction is thermodynamically favorable and hence spontaneous. The anodic redox potential is dependent upon the chemical nature of organic matter and can be calculated using the well-known Nernst equation. On the other hand, electrons in the cathodic chamber are commonly accepted by oxygen due to its availability and high redox potential (+0.82 V). Still, a number of other chemical acceptors are also used that includes nitrate, manganese oxide, iron, hydrogen peroxide, nitrite, etc. [Chaudhuri and Lovley, 2003]. The schematic representation of MFC is shown in Figure 2.3. The basic functional mechanisms of MFC would be clearer by an example of reaction on the electrode surface, as shown below:



The overall cell voltage can be described as:

$$E_{\text{cell}} = E_{\text{cathode}} - E_{\text{anode}} (+1.1V) \quad (2.3)$$

The ideal cell voltage that a system can generate is represented by equation 2.3, but due to the association of different losses in real MFC, the operating voltage is lowered. The detailed description of these different losses is described in section 2.8 below. Primarily, there are 3 integral components of a typical MFC, namely anode, cathode and proton/cation exchange membrane.

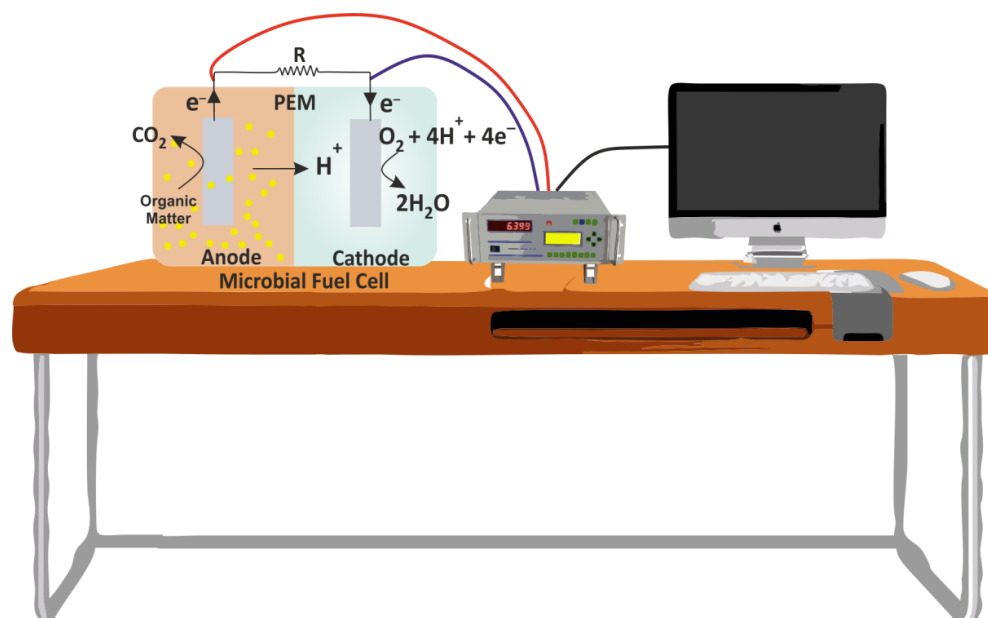


Figure 2.3: Schematic showing a typical MFC and its working principle.

2.6.1 Anode

The anode should have the following properties (i) corrosion resistance, (ii) high electrical conductivity, (iii) biocompatibility, (iv) high surface area, (v) chemical stability and mechanical strength [Guo et al., 2015; Rinaldi et al., 2008]. Carbon based conductive electrodes are frequently used in an anodic chamber. Classical examples include carbon paper, carbon brushes, carbon felt, reticulated vitreous carbon, graphite fiber brush, granular graphite, graphite plates, and rods, etc. [Hindatu et al., 2017; Zhou et al., 2011].

2.6.2 Cathode

MFC Cathode can be biotic or abiotic. Carbon based electrodes are the most preferred choice as a cathode as well. The abiotic electrodes generally have chemical/metal catalysts for acceptor reduction. Biotic electrodes, on the other hand, have algae/bacteria which aids both in acceptor reduction and production. Platinum based electrodes find applications in chemical fuel cells for high efficiency oxygen reduction at cathode [H. Liu et al., 2004a]. Pt based electrodes are not suitable for biotic cathodes because of several reasons. Pt is poisoned by phosphates, nitrates, and chlorides often used in the microbial growth medium. Pt is costly and also toxic to microorganisms. Non-platinum based catalysts like carbon nanotube, conductive polyaniline, metal oxide (lead oxide- PbO_2 , manganese (IV) dioxide), metals (cobalt and iron) serves well in biotic cathodes [He et al., 2017].

2.6.3 Membrane/ Separator

In order to maintain chemical equilibrium in the cell, usually, a membrane or separator is placed between anode and cathode which ensures the protons and/or cations transport from anodic to the cathodic chamber. The most commonly used membrane in conventional MFCs is Nafion 117. It is resistant to biofouling, has high ionic conductivity, impermeable to oxygen and organic acids [Logan and Regan, 2006c]. Its employability is limited by its high cost. In addition to this, researchers have used glass fibers, J-cloth, earthenware, nylon fibers, ceramics, and biodegradable shopping bags as alternative membrane separators [Santoro et al., 2017]. Table 2.3 is summarizing the membranes or separators employed in MFCs.

Table 2.3: Summary of different separators used in MFCs.

Membrane/ Separator	Base material	Proton conductivity	Power density (mW/m ²)	Reference
SPEEK	Sulfonated poly (ether ether ketone)	0.163×10^{-2}	77	[Narayanaswamy Venkatesan and Dharmalingam, 2015]
SPEEK/GO	SPEEK/Grapheme oxide composite	2.55×10^{-3}	41.70	[Shabani et al., 2019]
PES/SPEEK	Sulfonated poly (ether ether ketone)/poly (ether sulfone)	2.56×10^{-5}	170	[Lim et al., 2012]
SPEEK/SiO ₂	SPEEK/SiO ₂	1.018×10^{-2}	1008	[Sivsankaran and Sangeetha, 2015]
SPEEK/TiO ₂	SPEEK/TiO ₂ composite	0.187×10^{-2}	98.1	[Narayanaswamy Venkatesan and Dharmalingam, 2015]
SPES/PES	Sulfonated polyether sulfone/polyether sulfone	-	59	[Zinadini et al., 2017]
PS/SPEEK	(Polysulfone)/(sulfonated poly ether ether ketone)	-	97.47	[Ghasemi et al., 2016]
Nafion-112	Perfluorinated membrane	4.8×10^{-2}	19.7	[Ilbeygi et al., 2015]
Nafion-117	Perfluorinated membrane		106.7	[Ghasemi et al., 2013]
PVA/Nafion/ borosilicate	Polyvinyl alcohol-Nafion-borosilicate	0.07	-	[Tiwari et al., 2016]
Flemion	Fluorinated membrane mfg. by Asahi Glass Company, Japan	-	200	[Hosseini and Ahadzadeh, 2012]
PVDF-g-PSSA	poly(-vinylidene fluoride) grafted sodium styrene sulfonate	0.046	147	[Xu et al., 2019]
UF-1kDa	Ultra filtration membrane	-	36	[Hou et al., 2011]
Ceramic	Clay	-	5.23 W/m ³	[Jadhav et al., 2016]
Ceramic	Terracotta	-	400mW	[Ieropoulos et al., 2016]
Ceramic	Fine fire clay	-	2.1 mW	[Merino-Jimenez et al., 2017]
Ceramic	Mullite & terracotta	-	27 W/m ³	[Tremouli et al., 2018]

2.7 ELECTROCHEMICALLY ACTIVE MICROORGANISM

MFCs rely on the activity of electrochemically active/exoelectrogenic bacteria which transfer electrons extracellularly to conductive non-living electrodes. Studies have revealed three different mechanisms of electron transfer. This includes electron transfer by mediators or shuttles, by bacterial nanowires or by direct electron transfer. The examples of exoelectrogenic or exoelectrogens includes *Rhodospirillum rubrum*, *Shewanella putrefaciens*, *Geobacter metallireducens* and *Geobacter sulfurreducens* [Logan and Regan, 2006a; Lovley, 2006].

Small molecular weight mediators supplemented at cathode or produced inherently undergo cyclic oxidation-reduction shuttling the electrons between microorganisms and electrode. Microorganisms like *Pseudomonas aeruginosa*, *P. alcaliphila* and *Shewanella oneidensis* use this mechanism of electron transfer [Costa et al., 2018; Rabaey et al., 2004]. Lovley et al., 2005, discovered specialized structures called nanowires produced by bacteria when associated with electrodes [Reguera et al., 2005]. Direct electron transfer was discovered in *S. putrefaciens* by Kim et al. in 1999 [Kim et al., 1999]. Membrane c-type cytochromes transfer the electron to the electrode in direct electron transfer. Figure 2.4 is showing the different electron transfer mechanisms.

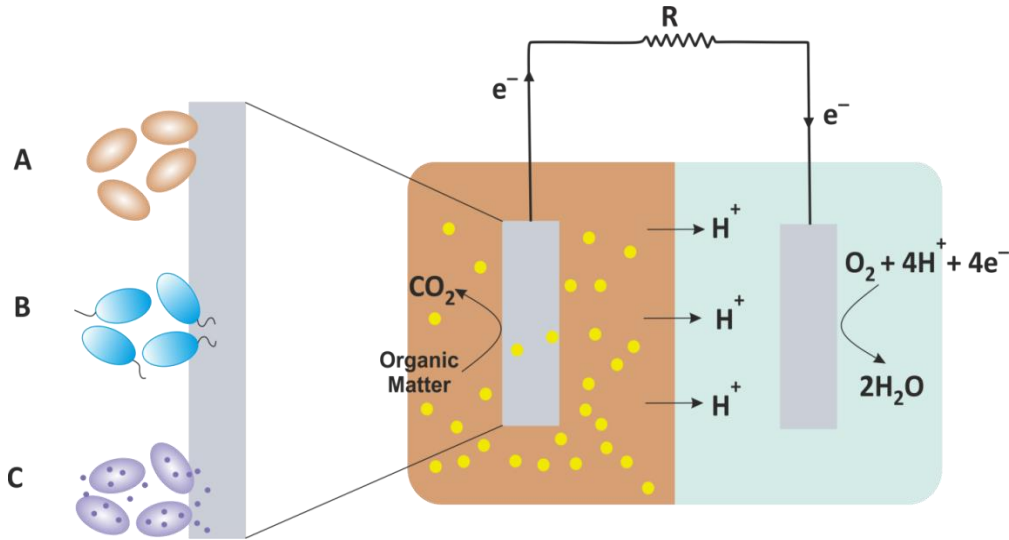


Figure 2.4: Illustration showing different electron transfer mechanism A) direct electron transfer, B) electron transfer by nano-wires, C) electron transfer through mediators.

2.8 DIFFERENT TYPES OF LOSSES IN MFC

The actual cell potential realized in MFC is lower than the theoretical potential due to several losses occurring at different steps of electron transfer (Figure 2.3). The operating voltage is calculated using equation 2.4 [Appleby and Foulkes, 1988]:-

$$V_{cell} = E_{cathode} - |\eta_{act,c} + \eta_{conc,c}| - E_{anode} - |\eta_{act,a} + \eta_{conc,a}| - iR_i \quad (2.4)$$

$\eta_{act,a}$ and $\eta_{act,c}$ are activation polarization losses on anode and cathode, respectively. On the other hand, $\eta_{conc,a}$ and $\eta_{conc,c}$ are concentration polarization in anodic and cathodic chamber, respectively.

The activation losses arise due to the sluggish electrode kinetics and complex electrochemical reactions occurring on the electrode surface. These can be minimized by adding the electroactive mediators, using efficient catalysts, increasing the electrode surface area & the operating temperature, or promoting the enriched biofilm on the electrode surface [Logan et al., 2006]. Concentration losses occur due to the slow mass transfer when the substrate concentration becomes limiting. This limits the current production in the MFC system. Concentration losses can be minimized by increasing the mass transfer rate of reactive species to/from the electrode by stirring or bubbling the solution. The third component is ohmic losses (η_{ohm}) which occur due to the resistance in the flow of ions present in the electrolyte and resistance in the flow of electrons from the electrode. It is expressed as iR_i in which i , is current flowing in the MFC system and R_i is the total internal resistance of the system. Ohmic losses can

be minimized by reducing the electrode spacing, or increasing the solution conductivity [Hoogers G, 2002].

2.9 ELECTROCHEMICAL TECHNIQUES AND PARAMETERS TO STUDY MFC PERFORMANCE

Various electrochemical parameters and techniques can assess the performance of MFC. These techniques used for characterizing MFCs are briefly described below:

2.9.1 Voltage and Power Density Measurement

Voltage and power values directly measure MFC performance. The open circuit voltage (OCV) is the voltage value when anode and cathode are not connected/at infinite resistance.

Open Circuit Potential (OCP) is the potential of cathode/ anode measured against a reference electrode like saturated calomel electrode (0.242V v/s the SHE), silver-silver chloride (Ag/AgCl) electrode (+0.197 V v/s SHE) or by standard hydrogen electrode (SHE) (0V). Power (Watts) is calculated by connecting anode and cathode via appropriate load/resister using the formula:

$$P = IE_{cell} \quad (2.5)$$

Where E_{cell} is the voltage of the system in volts and I is the current in Amperes. It can be calculated using the Ohm's law-

$$I = \frac{E_{cell}}{R} \quad (2.6)$$

Where R is the external resistance in Ohms applied to the MFC.

In order to calculate power density, power (Watt) is divided either by reactor volume (W/m^3) or electrode projected surface area (W/m^2). The polarization curve is a plot of voltage v/s current density. The system moves away from equilibrium during polarization. Resistance values are varied from high to low. Higher resistance values support high voltage and low current while low values support low voltage and high current. The current value at zero voltage is called short circuit current. At a particular value of resistance, voltage, and current are optimum and their product (power) is the highest. The midpoint on a hyperbolic curve points to the optimum power density and a suitable load/resistance a system can take. The polarization curve can be obtained by manually changing the resistance or using a potentiostat, which draws a fixed current and measures the output voltage by stepping up the load on the system. The three losses (mentioned in section-2.9) can be estimated by analyzing the polarization curve [Hoogers G., 2002].

2.9.2 Columbic Efficiency

Columbic efficiency is defined as the ratio of the total coulombs transfer to the anode from the substrate oxidation to maximum possible coulombs if all the substrate produces current [Logan et al., 2006]. The columbic efficiency can be calculated by the given equation:-

$$\varepsilon = \frac{M \int_0^t I dt}{FnV_{An} \Delta COD} \quad (2.7)$$

Where, M= 32 (molecular weight of oxygen), n= 4 (the number of electrons exchanged per mole of oxygen), F is Faraday's constant, V_{An} is the volume of the anodic chamber, and ΔCOD is the difference in COD over a time period.

2.9.3 Cyclic Voltammetry

Cyclic voltammetry (CV) measurement is the study of redox reaction occurring at the surface of electrodes. In this potentiodynamic electrochemical measurement, the current response is recorded when voltage in a particular potential range is ramped with time. CV is used to

elucidate electron transfer mechanisms between microorganisms and electrode [Fricke et al., 2008; Rabaey et al., 2004]. The CV is recorded generally in a three-electrode system with a working electrode (anode or cathode), a reference electrode, and a counter electrode (anode or cathode).

2.9.4 Chronoamperometry

Chronoamperometry (CA) helps in determining the reaction kinetics, diffusion coefficient, and reaction mechanism. This method is also useful for measuring biofilm growth and its activity on the electrode surface. To obtain a CA curve, the voltage of the working electrode is ramped, and the current response is recorded as a function of time. The process of the current generation and electron transfer is facilitated by biofilm formation at the electrode surface [Rosenbaum et al., 2007].

2.9.5 Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy (EIS) is an accurate method to measure ohmic, charge transfer, and mass transfer resistance of MFC [Dominguez-Benetton et al., 2012]. EIS is a powerful technique in which the response of current is measured when an AC voltage of small amplitude is applied to either electrode or complete cell [Scott and Yu, 2015]. The data obtained from EIS can be represented by the Nyquist plot and Bode plot. In the Nyquist plot, the x-axis and y-axis are the real part (Z_{Re}) and the imaginary part (Z_{Im}) of the impedance ($Z(\omega) = Z_{Re} + jZ_{Im}$), respectively. EIS is analyzed using the most appropriate equivalent circuit, which may comprise the resistor, capacitor, and Warburg elements in series/parallel.

2.10 MFC CONFIGURATION

The MFC configuration refers to the arrangement of different components of MFC. A configuration that caters best to the specific application sought and that offers minimum internal resistance is desirable. The parameters that lower the internal resistance and enhance the power output include a lesser distance between anode and cathode, less number of chambers, separator position & conductivity, electrode with high surface area, low effective volume, adequate mixing, etc. The prominent configurations include, single chamber, dual chamber, tubular, and stacked MFCs. Single chamber MFCs have a simple design and used when the aim is to remove COD and produce power. Atmospheric oxygen acts as the electron acceptor on an air-exposed cathode. On the other hand, Two/dual chamber MFC/ H shape systems are widely used and cater to several applications. The two chambers are physically separated by proton exchange membranes like Nafion or salt bridge [Logan et al., 2006; Logan and Regan, 2006b]. The utility of such reactors is limited to lab scale as these are difficult to scale up and realize relatively low power output, [Logan et al., 2006]. Furthermore, Tubular MFCs represents a further simplified design wherein a single long cylindrical/tubular chamber is used. Such systems are always membrane less and the height of the chamber ensures redox potential differences. The systems are often operated in an up flow mode wherein the feed containing high COD enters the bottom of the reactor and flows upwards while passing through the anodic and cathodic biofilms [Rabaey *et al.*, 2005]. The stacked MFC consists of multiple MFCs in either series or parallel or in a combination of both which generates a bio-battery with enhanced voltage/current output.

2.11 SCALED-UP STUDIES

Despite offering versatile advantages, the MFC technology is mostly restricted to lab scale only. The scales up studies are few. The reason is that the MFC scale-up leads to a substantial reduction in power output. The solution to lowered power output is to stack several small scale MFC units in series/parallel. The first trial of scaling up MFC was done at Foster's brewery in Yatala, Queensland (Australia), by the Advanced Water Management Center at the University

of Queensland. The study was conducted under the supervision of Jurg Keller and Korneel Rabaey. In this study, a total of 12 modules of 1000 L volume were used. Limited information is available on the performance of this MFC, except that the current generation was low [Keller and Rabaey 2008]. After this, several attempts have been made to bring this technology from lab to the field. Table 2.4 summarizes such large scale studies. The performance of the Scale-up MFC studies has given a mixed result with substantial power output in some and failure in others.

Table 2.4: Summary of prominent scaled-up studies in MFCs.

Volume (L)	Substrate	Operation mode	COD removal (%)	Power density W/m ² (W/m ³)	Reference
2.5	Manure slurry	Batch	-	0.03	[Scott et al., 2007]
2.7	Landfill leachate	Continuous	79	0.0018	[Gálvez et al., 2009]
2.7	Landfill leachate	Batch	79	NA	[Gálvez et al., 2009]
3.6	Sludge	Continuous	60	0.130(9.6)	[Ge et al., 2013]
4	Primary effluent	Continuous	65-70	NA	[F. Zhang et al., 2013]
10	Brewery	Continuous	86	0.093 (4.1)	[Zhuang et al., 2012]
20	Primary effluent	Continuous	80	0.38 (0.2)	[Jiang et al., 2011]
7.5	Synthetic	Continuous	81-97	(2)	[Clauwaert et al. 2009]
20	Synthetic	Continuous	-	1.44(144)	[Dekker et al., 2009]
250	Municipal wastewater	Continuous	79	116 mW	[Feng et al., 2014]
90	Brewery	Continuous	84.7	159	[Dong et al., 2015]
45	Municipal wastewater	Continuous	24	82	[Hiegemann et al., 2016]
240	Sediment	-	-	2.33 mW	[Ewing et al., 2014]
30	Urine	Continuous	-	2.6 V (Charged both basic & smart mobile)	[Walter et al., 2017]
85	Wastewater effluent	Continuous	79-82	0.1 (0.74)	[Rossi et al., 2019]
2.8	Dairy wastewater	Continuous	95	(0.48)	[Marassi et al., 2020]

2.12 APPLICATION OF MFC

Microbial fuel cells produce electrical energy but at significantly low output values (<6 W/m² Wm⁻²; ≤500 W/m³) as compared to chemical fuel cells which can generate power density of 140 kW/m³ [Arends and Verstraete, 2012; Zhao et al., 2009]. Therefore, MFCs cannot serve as standalone energy devices. The MFCs hold value when coupled with other applications like wastewater treatment, algae biomass generation, biosensors, powering remote sensors, bioremediation, carbon dioxide capture, etc. Some of the applications of MFC are discussed

briefly below:

2.12.1 Wastewater Treatment

Conventional wastewater treatment is energy intensive and integrating MFC systems to treat wastewater can offset the total energy requirements of the treatment process. It is estimated that 4-5% of the country's electrical energy is invested in water infrastructure including water treatment, water distribution, wastewater collection, and wastewater treatment. It is estimated that wastewater contains 9.3 times more energy than what is needed to treat water [Shizas and Bagley, 2004]. About 0.5 kWh/m³ of energy is required for the aeration process. It is also estimated that wastewater from domestic, animal, and food processes contain a total of 17GW of energy and can be used in MFC as a potential electron donor [Logan, 2008]. Other possible sources are textile industry, brewery wastewater, starch wastewater, landfill leachate, and cellulose-containing wastes [Franks et al., 2010; Scott and Yu, 2015]. When used for wastewater treatment, MFCs offer some specific advantages such as high COD degradation rate and lesser bacterial sludge when compared to activated sludge process [Hernández-Fernández et al., 2015]. It is estimated that the amount of sludge which needs disposal lowers by 50 to 90% using MFC [Holzman, 2005].

2.12.2 Electricity Generation

MFC converts chemical energy present in the organic matter into electrical energy. The electricity produced from MFC can be stored in the rechargeable devices such as a capacitor and can be used to power devices like wireless sensors [Shantaram et al., 2005] and robots [Ieropoulos et al., 2003, 2005]. MFCs can also power the implantable devices in the human body by utilizing nutrients supplied by the body [Rapoport et al., 2012].

2.12.3 Algae Cultivation and CO₂ Sequestration

Photosynthetic microbial fuel cell (PMFC) or algae assisted MFCs involves algae biomass production (at cathode) or degradation (at anode) or both. In these MFCs algae is introduced at the cathodic chamber as a source of oxygen, therefore eliminating the need for external aeration. In addition to this, algae biomass serves as a low cost readily available electron donor substrate at the anode. Algae also serve to treat water contaminated with nitrates and phosphates at the cathode [Zhang et al., 2011] and the CO₂ produced at the anode is fed to the cathode for capture by algal photosynthesis [Xiao and He, 2014]. Algae-assisted MFCs holds special promise as they aid in carbon capture, algae biomass generation, and easy operation [Rosenbaum et al., 2010; Zhang et al., 2011].

2.12.4 Bioremediation

Pollutants that are electron donors can be oxidized at anode while others that are electron deficient can be reduced at the cathode. The success of the process depends on the effectiveness of the microbial consortia which can be acclimatized for the given pollutant. Different electron donors such as textile dyes, petroleum hydrocarbons, phenolic compounds, ammonium compounds have been remediated at the anode [Pandey et al., 2016]. Similarly, metals such as mercury, arsenic, chromium, and ions such as nitrate, and sulfate can be reduced and precipitated at the cathode. Among the electron acceptors nitrate (NO₃/N₂ +0.74V), iron (Fe³⁺/Fe²⁺ +0.77V) and manganese (MnO₂/Mn²⁺ +0.60V) have a high reduction potential while sulfate (SO₄²⁻/H₂S -0.22V) has relatively low redox potential [Thauer et al., 1977; Rhoads et al., 2005, Al-Mamun et al., 2016].

2.12.5 Industrial applications

MFC is considered as an effective and efficient treatment technology for the effluents of several industries such as brewery, leather, molasses, distillery, textile, paper, dairy, food based and pulp etc. The last two decades have witnessed plenty of field applications by industries, companies and start-ups [Jadhav et al., 2021]. In this context, 16 modules of MFC units were

developed by an Israel based wastewater treatment company Emefcy [Doty, C. 2008]. Similarly, Lebone solution company (USA) developed a MFC model for the treatment of manure utilizing graphite cloth electrodes [Tweed, K. 2012]. In addition to this, a tubular MFC was commercialized and patented by a Netherlands based company namely, Plant-e B.V. The company employed this technology in plant-MFC application [Sudirjo et al., 2019]. There are several examples of industries such as Hy-syEnce (USA), Indian Oil Corporation Ltd. (India), Tata Consultancy Services (India), IntAct Labs LLC (USA) and few others, which are constantly trying to commercialize the MFC technology [Jadhav et al., 2021].

2.12.6 Electronics and sensor-based applications

In addition to nutrient recovery and wastewater treatment MFCs also being popularized in powering sensors and various electronic devices. Additionally, MFC has also proven its potential in in-situ and online monitoring of water quality on different parameters such as chemical/biological oxygen demand, heavy metal concentration, dissolved oxygen level, gas detection, and microbial activity assessment [Liu et al., 2014]. Based on these, MFC has been employed in many low-power sensors and electronic appliances such as LED bulbs, pH sensors, photo sensors and mobile charging, etc. [Kaur et al., 2013; Kim et al., 2013; Kim et al., 2003].

The MFC technology has also been explored in the field of robotics [Ieropoulos et al., 2012]. In this context, Ecobot-I was the first example of successfully demonstrated robot. It was powered by 8 units of MFCs. Similarly, the more advanced and automated version of Ecobot-I was Ecobot-II, comprising of 8 stacked MFC units [Melhuish et al., 2006]. All these above mentioned examples of self-sustainable MFC robots open new pathways for its application in underground sensors, space as well as remote areas.

2.13 PHOTOSYNTHETIC OR ALGAE ASSISTED MFCs

As mentioned earlier, algae assisted MFCs hold significant promise in making MFC technology sustainable. Algae-assisted MFCs can be powered by low-cost algae biomass; can produce algae biomass which serves the dual purpose of carbon capture, and oxygen generation. Oxygen is the most preferred electron acceptor in MFCs as it supports high potential differences. Algae cultivation at cathode provides the system with a continuous supply of oxygen (during the light period) and helps circumvent the installation of mechanical aerators. Figure 2.5 is illustrating the basic principle of algae assisted MFCs. Algae biomass also serves as feedstock for biodiesel generation and several other products as mentioned in the previous sections. Wang et al. reported a power density of 5.6 W/m³ in a *Chlorella* based MFC [Wang et al., 2010]. A culture of cyanobacteria, *Anabaena*, at cathode sparged with CO₂-air mixture gave a power density of 57.8 mW/m² [Pandit et al., 2012]. In one of the study, power density of 2.48 W/m³ and a CE of 9.4% were attained using immobilized algae systems [Zhou et al., 2012]. PMFC or algae assisted Microbial Fuel Cell or Microbial Carbon Capture Cell (MCCs) also serves as a modified photo bioreactor equipped with an inherent oxygen quenching mechanism and carbon dioxide supply. The process of algae cultivation at the cathode also complements the effective carbon removal at anode. Microalgae biomass is rich in hydrolysable carbohydrates, fats, and proteins and can serve as an electron donor substrate at anode [Cui et al., 2014].

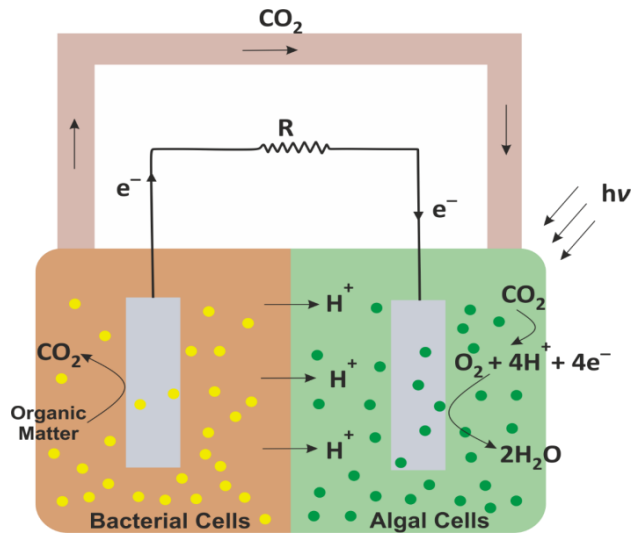


Figure 2.5: A typical algae assisted MFC showing power generation and algae cultivation.

2.14 Different configurations of algae assisted MFC

An algae assisted MFC can take different configurations depending on the intended application, i.e., algal production, power generation, wastewater treatment etc. The configuration varies from triple chamber to single chamber. Single chamber algae assisted MFC, involve bacterial and algae cultivation in the same chamber. The CO₂ produced by bacteria is effectively sequestered by the microalgae present in the same chamber. The carbon capture is efficient and system is easily maintainable. In a two chambered system, algae and bacterial consortiums are separated by a PEM. These systems are used for algae cultivation for bioenergy or other applications. A separate photo-bioreactor is sometimes coupled with the system to enhance algae growth rate and power generation. A three chamber algae-based MFC finds application in water desalination, where saltwater is fed to the middle chamber to facilitate flow of positive and negative ions. Researchers have also used uplift aeration type MFC to support high algae growth rates [Saba et al., 2017]. Various kinds of algae-based MFC configurations are shown in figure 2.6.

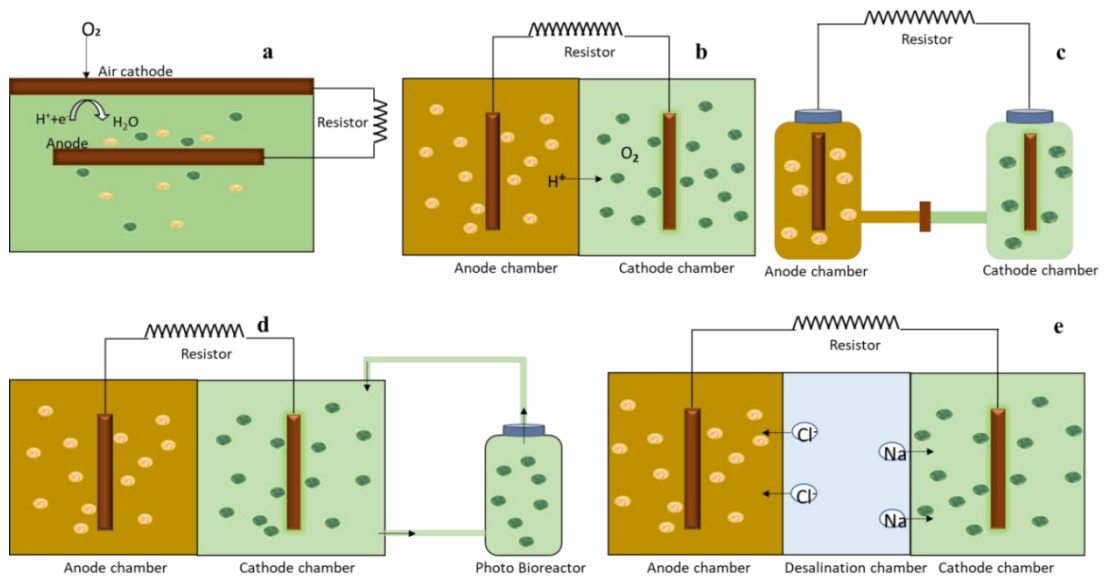


Figure 2.6: Schematic showing different algae assisted MFC configurations a) single chamber; b) Dual chamber; c) H-shaped; d) dual chamber integrated with external photobioreactor & e) three chamber with desalination.

2.15 ALGAE BIOMASS AS ANODIC SUBSTRATE

Algae biomass is rich in decomposable carbohydrates, lipids and proteins. Therefore, algae serve as a good source of electron donor at the anode. The primary challenge with the use of intact algae biomass is its complex cell wall. Algae cell wall composition varies from class to class and species to species. The chlorophycophyta contains a wide array of cell walls ranging from cellulose pectin complexes to hydroxyproline rich glycoproteins. Like plants cells, algae cell walls are intricate mix of polymers such as cellulose, hemicellulose, lignin, pectin, arabinogalactan proteins, extension etc. This complex assembly of polymers in algae cell wall necessitates the biomass pretreatment to break open the structure, enhance surface area, and hydrolyze of polymers. Researchers have used both intact and pretreated micro and macroalgae as anodic substrates and reported good power outputs [Velasquez-orta et al., 2009, Cui et al., 2014]. The use of pre-digested algae biomass also supports high power output over undigested biomass [Salar-garcía et al., 2016].

2.16 EMPLOYMENT OF ALGAE IN CATHODE

2.16.1 Algae at cathode

Algae at cathode not only serve as oxygen supplier but also as catalysts for oxygen reduction at electrode surface. Algae produced metabolites also serve as electron acceptors in the absence of oxygen particularly during the dark period. The success of algae assisted MFC depends on the process of photosynthesis which is driven by light energy and carbon dioxide supply [Elmekawy et al., 2014; González del campo et al., 2013, Hu et al., 2016b].

2.16.2 COD removal and wastewater treatment

Algae can grow in an autotrophic, heterotrophic, and mixotrophic mode. Heterotrophic and mixotrophic modes assist with carbon removal. Algae can effectively remove nitrates and phosphates from the water. The Simultaneous carbon, nitrogen, and phosphorus removal is possible using dual chamber algae assisted MFCs. Anode and cathode both can contribute towards carbon removal while algae assisted cathode can help with nitrogen and phosphorus removal (Table 2.2) [Commault et al., 2017]. The success of algae assisted MFC in wastewater treatment depends on algal strain, inoculum size, density, temperature, N/P ratio, salinity, pH, light intensity and CO₂ supply & capture rate. A algae assisted MFC thus needs optimization with respect to all these parameters [Reddy et al., 2019].

2.17 DIFFERENT ALGAL STRAINS USED IN ALGAE ASSISTED MFC

Researchers have used different types of algal strains in algae assisted MFC. Table 2.5 summarizes the performance of algae assisted MFCs with respect to algae species and configuration.

Table 2.5: Different strains of microalgae & the corresponding dissolved oxygen (DO) and power output obtained in algae assisted MFCs

Reactor configuration	Algae species	DO concentration (mg/L)	Power Density	References
Dual-chamber	<i>Chlorella</i>	-	3720 mW/m ³	[Zhang et al., 2019]
Single chamber	<i>Scenedesmus quadricauda</i>	-	62.93 mW/m ²	[Yang et al., 2018]
Dual- chamber	Mix culture	19.57	50 mW/m ²	[Nguyen et al., 2017]

Sediment MFC	Mix culture	14.2	22.19 mW/m ²	[Neethu and Ghangrekar, 2017]
Tubular	<i>Chlorella</i>	-	200 mA/m ²	[Ma et al., 2017]
Two chamber	<i>Spirulina</i>	-	0.85 W/m ²	[Colombo et al., 2017]
Air-lift type MFC	<i>C. vulgaris</i>	5.65	558 mW/m ³	[Hu et al., 2016a]
Two chamber	Mix culture	-	128 μ W	[Gajda et al., 2015]
Two chamber	<i>C. vulgaris</i>	-	34.2 mW/m ²	[Commault et al., 2017]
Two chamber	<i>C. vulgaris</i>	8.5	126 mW/m ³	[Bazdar et al., 2018]
Two chamber	<i>C. vulgaris</i>	-	1926 mW/m ²	[Cui et al., 2014]
Two chamber	<i>Microcystis aeruginosa</i>	-	58.4 mW/m ³	[Cai et al., 2013]
Two chamber	Mix culture	20.8	0.35 V	[Kakarla and Min, 2019]
Two chamber	<i>C. vulgaris</i>	12	14.40 mW/m ²	[González Del Campo et al., 2013]
Two chamber	<i>C. vulgaris</i>	100% saturated DO in water	23.97 mW/m ²	[Gonzalez del Campo et al., 2014]
Two chamber	<i>C. vulgaris</i>	7	42.98 mW/m ²	[Gonzalez Del Campo et al., 2015]
Two chamber	<i>C. vulgaris</i>	-	62.7 mW/m ²	[Gouveia et al., 2014]
Two chamber	<i>Chlorella sp. QB-102</i>	-	36.4 mW/m ²	[Y. Zhang et al., 2018]
Two chamber	<i>C. vulgaris</i>	-	327.67 mW/m ²	[Huarachi-Olivera et al., 2018]
Two chamber	<i>C. sorokiniana</i>	7	3.2 W/m ³	[Neethu et al., 2018]

2.18 POWER OUTPUT FROM ALGAE ASSISTED MFC

Power output from algae assisted MFC can be optimized by choosing an appropriate algae species, electrode material, catalyst coatings, chamber design, light duration & intensity, electron donor substrate, and CO₂ sources. The microalgae can directly generate current either by introducing it in the anodic chamber as an electron donor substrate or in the cathodic chamber as a biocatalyst for generation of oxygen [Elmekawy et al., 2014]. Table 2.5 summarizes the prominent studies in terms of power output obtained from different algal strains employed in algae assisted MFCs.

2.19 FACTORS AFFECTING POWER OUTPUT IN ALGAE ASSISTED MFC

2.19.1 Light

Light is the primary requirement for photosynthesis. Light intensity, its duration (light: dark period), and wavelength all affect algae growth. High light intensities lead to photo oxidation and growth inhibition. On the other hand, low intensities lower algae growth rates and promote bacterial growth. Both polychromatic and monochromatic light is used for cultivating algae. Amongst the monochromatic light, the red and blue light is most preferred for high rate algal culture. Light source and its orientation with respect to MFC affect algae growth. Researchers prefer using inbuilt LED lights that ensure direct illumination and minimize the self-shading effect. It also helps regulate temperature and ensure low temperatures enabling optimized algae growth. The ratio of light: dark period is also critical and varies from species to species and system to system [Saba et al. 2017; Reddy et al., 2019].

2.19.2 CO₂ concentration

Carbon dioxide is another key ingredient needed for algae growth. Most of the algae grow well at atmospheric CO₂ levels. However, higher concentrations are shown to promote algae growth and carbon capture [Singh and Singh, 2014]. Researchers have studied the impact CO₂ concentration on growth and lipid production in algae [Sato et al., 2003; Wang et al., 2010]. The response varies from species to species and also on the cultivation conditions. In a algae assisted MFC, the CO₂ required by the microalgae can either be the CO₂ present in the anodic off-gas [Wang et al., 2010] or it can be CO₂ sparged separately [González Del Campo et al., 2013]. However, CO₂ sparging is associated with certain disadvantages, including the lowering of pH on the dissolution of CO₂ in water, which can be resolved by the use of higher initial inoculum concentration [Chiu et al., 2009; Zhang et al., 2014]. Increasing the CO₂ concentration by 10-15 % has resulted in a 6% increase in the lipid content, proving the significance of CO₂ concentration in the lipid content of algal cells [J. Liu et al., 2011].

2.19.3 Dissolved oxygen

Photosynthesis liberates oxygen via light reaction and algae consume oxygen while respiring. A high concentration of oxygen becomes inhibitory for algae growth and leads to photo-oxidative damage. It was found that a DO concentration greater than 30 mg/l inhibited the *C. vulgaris* growth by 30% [Kazbar et al., 2019]. MFC circumvents this problem as oxygen is quenched through reduction reaction in a circuit MFC. The solubility of oxygen in water is also dependent on temperature, salt content, and duration of light/dark cycles. It is often observed that during night time, the DO level drops and so is the power output from MFC [Gonzalez del campo et al., 2015]. A DO level of 4.5-5.5 mg/l is suitable for supporting continuous power output from MFC [Rodrigo et al., 2010], while algae based cathode can realize DO level in the order of 6.6 mg/l [Kang et al., 2003]. Another major factor that determines the DO concentration in water is temperature. Hence the use of proper lighting equipment with useful wavelengths is of utmost importance. In other words, DO is dependent on the temperature as well as the duration of light/dark cycles. One important investigation to prove this relationship was the one carried out by Gouveia et al, wherein experiments were conducted using two light intensities 26 $\mu\text{E}/\text{m}^2$ and 96 $\mu\text{E}/\text{m}^2$. A ten times increase in algal growth rate with increased oxygen concentration resulting in enhanced power generation was observed in this investigation [Gouveia et al., 2014].

2.20 ALGAL BIOMASS GENERATION THROUGH ALGAE ASSISTED MFC

The algal biomass production in algae assisted MFC is important to assess the overall system performance and net energy recovery. The main factors which affect the algal growth in MFC include reactor configuration, wastewater composition and light intensity [Luo et al., 2017]. Despite some obvious advantages, the algae growth rates and productivities achieved in MFCs are low. This is primarily due to the lack of studies specifically investigating the algae growth rate in MFCs and on system scale up. The table 2.6 is summarizing the key studies reporting the COD removal by particular algal strains and their net biomass production.

Table 2.6: Summary of COD removal and algal biomass generation in algae assisted MFCs.

MFC type	Substrate or Nutrient media used in cathode	Algal strain	Removal efficiency (%)			Biomass concentration (mg/l)	References
			COD	TN	TP		
Double chamber	Synthetic media	<i>Chlorella sp. QB-102</i>	-	-	-	-	[Y. Zhang et al., 2018]
Double chamber	Landfill leachate wastewater	Mixed culture	52.8	80	-	-	[Nguyen et al., 2017]

Double chamber	Synthetic media	Mixed culture		100			[Kakarla and Min, 2019]
Dual chamber integrated with photobio-reactor	CO ₂	Mixed culture				470	[Gajda et al., 2015]
Dual chamber	CO ₂	<i>Chlorella vulgaris</i>	80			360	[González Del Campo et al., 2013]
Single chamber	CO ₂	<i>Chlorella vulgaris</i>	44			270	[Q. Hou et al., 2016]
Dual chamber	Chocolate factory	<i>Chlorella vulgaris</i>	78.6			5.2	[Huarachi-Olivera et al., 2018]
Single & dual chamber	Synthetic media	<i>Spirulina</i>	89	5.5	17		[Colombo et al., 2017]
Dual chamber	Anodic effluent	<i>Chlorella vulgaris</i>	49	83			[Commault et al., 2017]
Dual chamber	CO ₂ from anode chamber	<i>Chlorella vulgaris</i>	90			1247	[Cui et al., 2014]
Dual chamber	Externally supplied CO ₂	<i>Chlorella vulgaris</i>	5.5			3600	[Bazdar et al., 2018]
Dual chamber	CO ₂ from anode chamber	<i>Scenedesmus acutus</i> <i>PUVW12</i>	87			290	[Angioni et al., 2018]
Single chamber	Anaerobically digested kitchen waste effluent	<i>Golenkinia</i> sp. SDEC-16, <i>Scenedesmus</i> SDEC-8 & <i>Scenedesmus</i> SDEC-13	43.6	38	100	325	[Q. Hou et al., 2016]

Considering the advantages of using MFC for algae cultivation, the promises associated with algae based products, the energy producing capability of MFCs and the need to scale up the system, the study was undertaken with the following objectives: -

1. To develop a closed-loop self-sustainable MFCs wherein algae biomass generation at the cathode is complemented by algae biomass degradation at the anode.
2. To scale up the MFCs using low-cost materials and operation in outdoor conditions.
3. To develop a suitable electrode catalyst for algae-based cathodes.

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