

Assessing the factors influencing the performance of constructed wetland–microbial fuel cell integration

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ABSTRACT

Constructed wetland coupled microbial fuel cell (CW-MFC) systems integrate an aerobic zone and an anaerobic zone to treat wastewater and to generate bioenergy. The concept evolves based on the principles of constructed wetlands and plant MFC (one form of photosynthetic MFC) technologies, of which all contain plants. CW-MFC have been used in a wide range of application since their introduction in 2012 for wastewater treatment and electricity generation. However, there are few reports on the individual components and their performance on CW-MFC efficiency. The performance and efficiency of this technology are significantly influenced by several factors such as the organic load and sewage composition, hydraulic retention time, cathode dissolved oxygen, electrode materials and wetland plants. This paper reviews the influence of the macrophyte (wetland plants) component, substrate material, microorganisms, electrode material and hydraulic retention time (HRT) on CW-MFC performance in wastewater treatment and electricity generation. The study assesses the relationship between these parameters and discusses progress in the development of this integrated system to date.

Key words | constructed wetland, electricity generation, microbial fuel cell, wastewater treatment

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INTRODUCTION

According to the current Sustainable Development Goals (SDGs) which focus on a ‘win-win’ situation, for environmental and economic goals to be complementary requires the use of more sustainable means of energy generation and wastewater treatment measures (Williams 2000; UN 2015). In 1911, Michael C. Potter studied and proposed the first microbial electrochemical technology (MET) and bio-electrochemical system (BES) known as microbial fuel cells (MFCs) as sustainable biotechnology (Potter 1911). In the last ten years, the microbial fuel cell has been extensively studied worldwide, and today, it is recognized as a sustainable biotechnology for both electricity generation and wastewater treatment (Virdis *et al.* 2011; Singh *et al.* 2019). MFC technology provides a new method to offset wastewater treatment plant operating costs and energy demand by extracting energy inherent in wastewater while treating wastewater simultaneously. This makes wastewater treatment more affordable and ecologically friendly for both developing and industrialized nations, which is highly consistent with the aim of sustainability (Liu *et al.* 2004).

However, most recently, researchers also found that the naturally existing stratified redox gradients in constructed wetlands (CWs) are highly consistent with the conditions in MFCs, i.e. aerobic zone at the air–water interface and anaerobic area in the inner/lower part. This makes their integration very plausible by creating a synergy for the treatment of wastewater and energy generation sustainably. Through this integration, a newly merged technique was formed which is called constructed wetland–microbial fuel cell (CW-MFC) (Yadav *et al.* 2012; Doherty *et al.* 2015a, 2015b). The performance and output efficiency of this newly evolved technology is controlled by the magnitude and conditions of certain component factors before and during its operations. Therefore, the appropriate selection of certain key component factors is paramount in the design and construction of CW-MFC systems. Design performance is influenced significantly by the choice of wetland plant species, substrate material, microbial activities, electrode material, and hydraulic retention time along with other variables of fundamental importance such as flow regime for the success of this

technology (Shi *et al.* 2018). Earlier and recent reviews by Doherty *et al.* (2015a, 2015b; Shi *et al.* 2018) and Srivastava *et al.* (2019) on CW-MFC focused on different aspects of the technology. Doherty *et al.* (2015a, 2015b), focused on the performance of CW-MFCs functioning as CW regarding wastewater treatment and CW-MFCs functioning as MFC regarding electricity generation, whereas Srivastava *et al.* (2019) also focused on constructed wetland coupled microbial fuel cell technology, development and potential applications. However, this paper reviews the role of the wetland plant component, the electrode material, the microbial activities, substrate material and the hydraulic retention time of CW-MFC systems and their effect on power generation and wastewater treatment. This review aims at summarizing the current situation with regard to these essential components, investigating the potential of the technology and guiding future research, in the selection of appropriate design materials.

BASIC CONCEPT OF CW-MFC INTEGRATION

Microbial fuel cell technology has a dual advantage of wastewater treatment and electricity generation. Research efforts have been made to improve its power output. However, MFC seems limited at the pilot scale and power outputs appear to have plateaued. As such, some integrated technologies have emerged based on MFC. These hybrid technologies have the larger potential for scaling up and practical application compared with the pure MFC (Xu *et al.* 2015). The MFC hybrid treatment system was proposed as an example of integrating MFC and relevant wastewater technologies for improving treatment sustainability and energy generation (Das 2018).

Recent studies reveal constructed wetlands and microbial fuel cell technologies as possible technologies that can be merged as a hybrid system (Yadav *et al.* 2012). This is based on the fact that constructed wetlands have an aerobic surface layer and an anaerobic lower layer which establishes a naturally existing stratified redox gradient which is similar to an MFC's redox reactions in a single of its two-chambered cell (Fang *et al.* 2017). Again, the MFC's bio-anode is capable of wastewater treatment, which has been tested in diverse cases. This phenomenon, therefore, makes CW-MFC integration meaningful and feasible. In the integrated system, a conductive material is embedded into the lower layer of a CW (as the anode) and another in the surface layer (as the cathode), which can simultaneously treat wastewater and generate bioelectricity (Doherty *et al.* 2015a, 2015b), as shown in Figure 1. This hybrid system, CW-MFC, seeks for the integration of MFC in constructed wetlands by using the triple synergy of the physical, chemical and biological aspects of the substrate, plants and microorganisms for wastewater treatment and electricity generation (Yang *et al.* 2018a, 2018b). The CW-MFC hybrid system combines the advantage of the two systems in an economical and effective way capable of achieving high levels of wastewater treatment and bioenergy.

Since its introduction, numerous studies have been carried out to improve the efficiency and scale-up of this technology. However, prior to that, chemical oxygen demand (COD) was typically used to demonstrate the ability of the integration (the hybrid system) further to improve COD removal efficiency from wastewater as compared with MFC or CW standalone (Wang *et al.* 2017a, 2017b). Srivastava *et al.* (2015) found that the CW-MFC integration contributes to 27–49% COD removal (0.5 g/L–0.75 g/L glucose load) in a hybrid system compared with traditional CW,

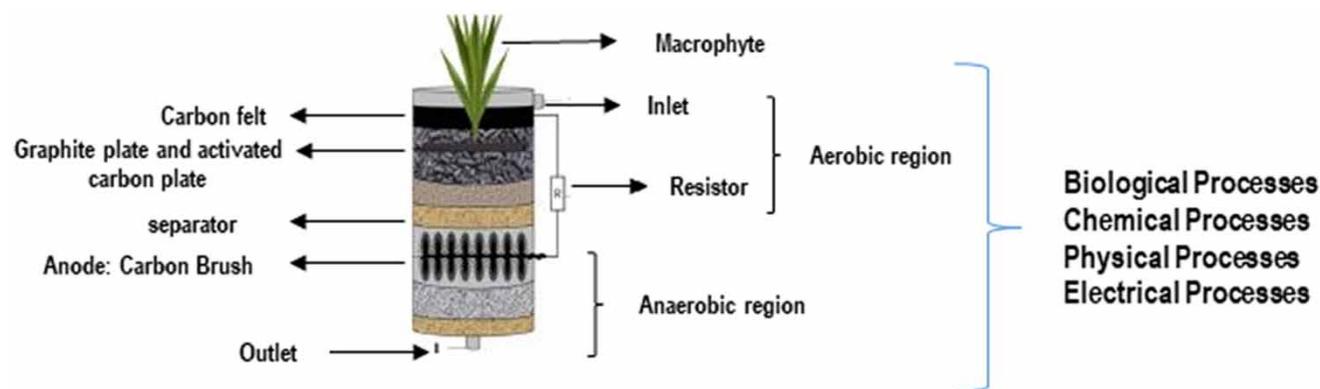


Figure 1 | Schematic representation of constructed wetland integrated with microbial fuel cell.

while this value in other work done by Fang *et al.* (2013) was 12.6% (using bio-recalcitrant azo dye (ABRX3) as the substrate with a COD of 180 mg/L). Similarly, it was found that 33% of the total COD was removed in the anode zone, which only resided in 13.6% of the liquid volume of the CW-MFC.

CW-MFC CONFIGURATION AND OPERATION

At the laboratory scale, the CW-MFC reactor is usually made with acrylic/polyacrylic or polyvinyl chloride (PVC) tanks with drilled inlet and outlet holes for influent and effluent respectively. Araneda *et al.* (2018) constructed a CW-MFC reactor tube using an acrylic column, whereas some other researchers like Yang *et al.* (2018a, 2018b) and Xu *et al.* (2018a) used materials like polyvinyl chloride and Perspex containers respectively, with varying dimensions. However, the designs and dimensions vary based on their study scale. Other researchers have used materials such as polycarbonate plastic cylinders and polyester chambers (Liu *et al.* 2013; Saz *et al.* 2018).

A typical CW-MFC like a microbial fuel cell has two main regions: the anaerobic region and the aerobic region respectively containing the anode and the cathode materials acting as electrodes (Araneda *et al.* 2018; Yang *et al.* 2018a, 2018b). The electrodes are connected using titanium wires with an external resistance to close the circuit for electricity generation. Carbon and graphite are commonly used as electrode materials since they offer long-term sustainability owing to their high electrical conductivity, non-oxidative nature and the fact that they provide a good medium for the attachment and growth of a microbial community (Doherty *et al.* 2015a, 2015b; Yadav *et al.* 2018). The lower anaerobic region is often separated from the upper cathodic region by the use of separators, to keep the electrodes apart. Such an arrangement is done to create a sharp redox gradient in the system needed for microbial reactions and electron transfer. The first CW-MFC constructed by Yadav *et al.* (2012), under an up-flow regime, employed glass wool as a separator and some other studies from Villaseñor *et al.* (2013), Doherty *et al.* (2015a, 2015b) and Yang *et al.* (2018a, 2018b), similarly used the same material or a bentonite layer as a separator. However, in recent studies, the use of separators has been avoided due to the system's susceptibility to clogging and overall increase in the internal resistance of the system and thus lower bioelectricity generation when separators are used. In a study conducted by Xu *et al.* (2018a, 2018b) to assess the influence of glass wool (GW) as separator on bioelectricity generation in a CW-MFC,

their results showed that the highest voltage was achieved in the non-separator (NS) system (465.7 ± 4.2 mV with electrode spacing of 5 cm), which was 48.9% higher than the highest value generated in the GW system (312 ± 7.0 mV with electrode spacing of 2 cm). And the highest power density was produced in the NS system (66.22 mW/m²), which was 3.9 times higher than the value in the GW system (17.14 mW/m²).

In addition, the configuration of a CW-MFC reactor like a characteristic constructed wetland has its lower anaerobic compartment filled with a layer of gravel or soil as a supporting substrate for the anode electrode and the oxidation reaction required for the removal processes and electron transfer by the microbial community (Yang *et al.* 2018a, 2018b). The upper cathode compartment is planted with macrophytes, which supply dissolved oxygen to the cathode via plant root respiration, which contributes greatly to the reduction reaction. Macrophytes also play a key role in CW-MFC as biofilters and accumulators in the treatment process. According to Fang *et al.* (2013) the presence of plants promotes oxygen concentration in the cathode through their photosynthesis. A CW-MFC with plants generated an average voltage output about 15% higher than unplanted CW-MFC.

MAJOR COMPONENT FACTORS AFFECTING THE PERFORMANCE OF CW-MFC

The performance and output efficiency of every technology is controlled by the magnitude and conditions of certain factors and components before, during and after the operation of the technology. Studies have reported the influence of numerous component factors that significantly affect the performance of this hybrid technology, such as: wetland macrophyte, substrate and electrode material, microorganisms and hydraulic retention time (HRT). Details of their mechanism, how they affect performance and their relationship with each other are explained in detail in the sections below.

WETLAND MACROPHYTE (PLANT)

Aquatic macrophytes are aquatic plants large enough to be visible to the naked eye. They grow in water or wet areas (Brix & Schierup 1989). Some are rooted in the sediment, while others float on the water's surface and are not rooted in any substrate. This varying growth location gives rise to four types of macrophyte classification: the submerged, the emergent, the free-floating and floating leaved macrophytes

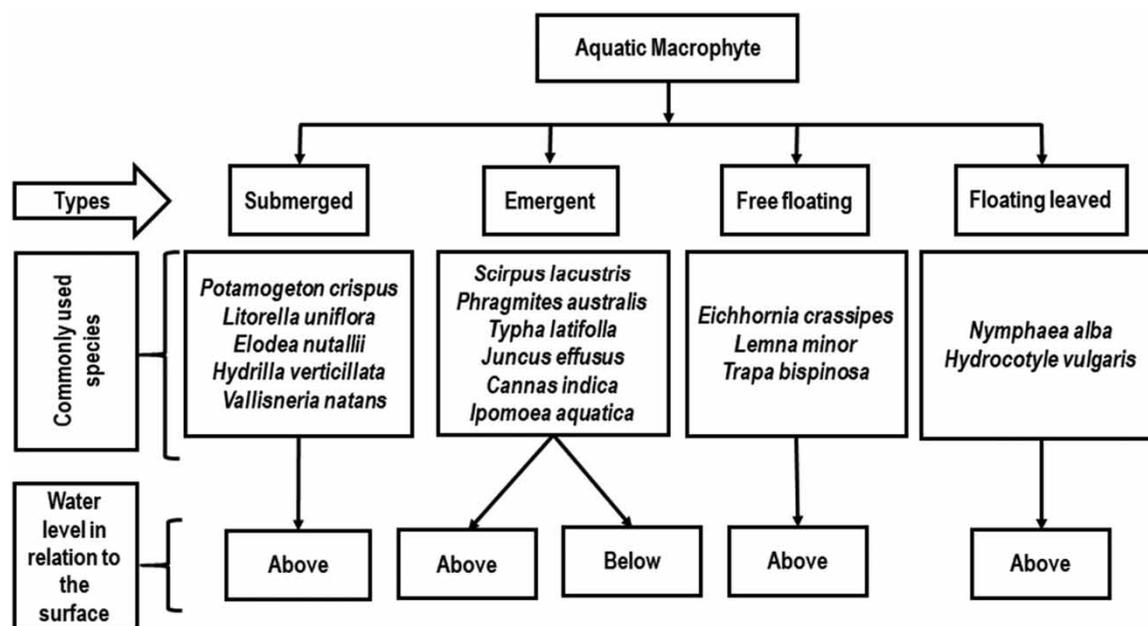
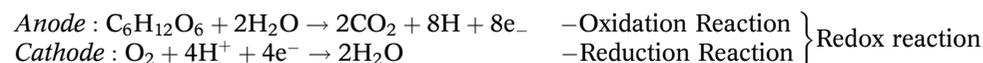


Figure 2 | Schematic representation of the different types of macrophytes and the most commonly used species in CW-MFC.

(Brix & Schierup 1989; Vidal 2017), as shown in Figure 2. The species of macrophytes used in CW-MFC are crucial to the success of the system. Various studies have considered certain selection criteria with the aim of enhancing performance, which includes the following criteria. (i) Good natural adaptation to the local climate; Aguirre Sierra (2017) concluded that native species are best preferred. In that study, the most common aquatic plants in the region proved to give better performance in the CW-MFC (Aguirre Sierra 2017; Oodally et al. 2019). (ii) Rapid growth and high biomass production. (iii) Nutrient absorption capacity. (iv) Adaptation and ease of propagation. (v) Good root development: in a sediment microbial fuel cell (SMFC) with wetland plant experiment conducted by Chen et al. (2012), their experiment revealed that young roots were able to excrete more oxygen

THE ROLE OF MACROPHYTE IN REDOX REACTIONS

In MFC, electricity production is basically the result of oxidation and reduction reactions which release (the anode) and accept (cathode) electrons within a biochemical or electrochemical system. One acts as an electron donor while the other essentially serves as an electron acceptor (Gude 2016). The chemical compounds that are responsible for accepting electrons are called terminal electron acceptors (TEA). In this system, materials such as transitional metals or nitrate can also be used as electron acceptors (Clauwaert et al. 2007; Jadhav et al. 2014). However, oxygen is commonly used owing to its inherent sustainability and comparatively high redox potential (Xu et al. 2015), for example in the case of the organic matter (substrate) and oxygen as the electron acceptor.



than mature or aging roots. This indicates that the maturity of the root and its development is an important factor to consider in macrophyte selection. (vi) Oxygen transfer capacity to the roots by creating an aerobic environment.

However, due to the great diversity of flora, further research is needed concerning the evaluation and selection of plant species having potential for use in CW-MFC for simultaneous wastewater treatment and electricity production (Vogelmann et al. 2017).

The need for oxygen in the upper cathodic compartment to aid the reduction reaction makes the plant component in the CW-MFC an important part of the system's performance and efficiency (Yadav et al. 2018).

Likewise, macrophytes play a very essential role in the cathode region by making oxygen available. As shown in Figure 3, they excrete oxygen through their rhizosphere during photosynthesis. The air-cathode at the air-water interface was used by some researchers to increase the

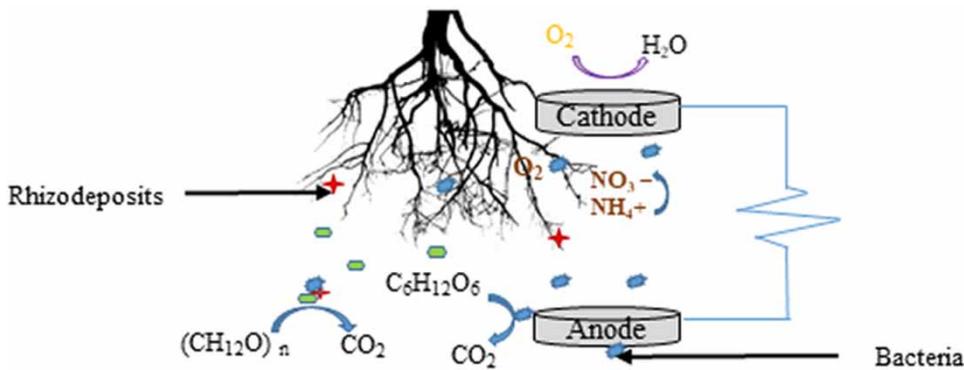


Figure 3 | The principal reactions for the overall process of the CW-MFC. (1) Plant roots release O_2 and excrete $(\text{CH}_2\text{O})_n$. (2) Electrons are produced at the anode from the anaerobic degradation of $(\text{CH}_2\text{O})_n$ and $\text{C}_6\text{H}_{12}\text{O}_6$. (3) NH_4^+ is converted to NO_3^- by nitrifying bacteria in the rhizosphere. (4) O_2 and NO_3^- are electron acceptors at the cathode. (5) Electricity production.

cathodic reaction by harnessing the dissolved oxygen at the air–water interface (Doherty et al. 2015a, 2015b). However, the use of the air-cathode was recognized to increase the spacing between the anode and the cathode and hence increase the internal resistance (Chen et al. 2012; Shi et al. 2018). This distance can be reduced by exploiting the oxygen excreted by the roots of the macrophyte. In an experiment designed by Fang et al. (2013) comparing planted and unplanted CW-MFC, the plants promoted the oxygen concentration in the cathode through their rhizosphere. Also, the average voltage of the planted CW-MFC was 15% higher than that of the unplanted. Liu et al. (2013) also developed a constructed wetland coupled with MFC on the principles of the photosynthetic MFC by utilizing root exudates of *Ipomoea aquatica* as part of the fuel. They achieved a maximum power density of 12.42 mW/m^2 produced from the CW-MFC planted with *I. aquatica*, which was 142% higher than the 5.13 mW/m^2 obtained from the CW-MFC without plants.

ROLE OF MACROPHYTE IN TREATING WASTEWATER

Another important role of the plant component in CW-MFC is the role macrophytes play in wastewater treatment. Generally, treatment in CW-MFC takes place when the wastewater flows through the different sections (levels) of the artificial wetlands, where physicochemical and biological processes such as filtration, sedimentation, adsorption, bioaccumulation and denitrification by the microbial community occur. Soil or gravel substrate and plant macrophyte produce a treated effluent. These processes mimic the exact processes that occur in natural wetlands

in wastewater treatment for the removal of contaminants (Aguirre Sierra 2017). However, the plant component is recognized as a versatile player in the treatment effect.

Besides the release of oxygen, which influences the redox potential, the root system of the submerging plant releases exudates or carbon compounds through a process called ‘rhizodeposition’ that act as a form of carbon source for denitrifiers for the removal of nitrate (Doherty et al. 2015a, 2015b; Aguirre Sierra 2017). In addition, wetland plants play an essential role in supporting a wide range of microbes by providing an attachment surface for degradation by bacteria. Through their root structure, they also have the filtering and adsorption effect needed for the removal of contaminants (Brix & Schierup 1989). Liu et al. (2016) reported that wetland plant species have a major effect on pollutant removal efficiencies. In the study of Liu et al. (2013), the root exudates of *Ipomoea aquatica* were utilized as part of the fuel in a photosynthetic MFC. Liu et al. (2013) reported that wetland plant species have a major effect on pollutant removal efficiencies as well as on the microbial communities. *Phragmites australis* had better removal of $\text{NH}_4\text{-N}$ than *Iris pseudacorus* and can enhance the nitrification process in the rhizosphere as a result of stronger radial oxygen loss (ROL). Furthermore, plants may uptake pollutants such as N, P and heavy metals; however, several studies have reported that plant uptake is inappreciable with respect to heavy metals (Vymazal 2010; Brix & Schierup 1989). In a recent study by Oon et al. (2017) using an *Elodea nuttallii* CW-MFC under artificial aeration, they achieved 98% COD removal efficiency (Table 1). Saz et al. (2018) also assessed the effect of vegetation on treatment performance and electricity in CW-MFC comparing four species; *Typha latifolia*, *Typha angustifolia*, *J. gerardii* and *C. divisa*. At the end of their experiment, *Typha angustifolia* recorded the

Table 1 | Comparison of COD removal efficiency and power density between different macrophyte based on earlier studies

Plant species	HRT (h)	COD removal efficiency (%)	Maximum power density (mWm ⁻²)	Reference
<i>Canna indica</i>	96	75	15.73	Yadav et al. (2012)
<i>Ipomoea aquatica</i>		85.7	5.62	Fang et al. (2013)
<i>Phragmites australis</i>		90.0–95.0	20.76	Villaseñor et al. (2013)
<i>Phragmites australis</i>		76.5	12.37	Zhao et al. (2013)
<i>Ipomoea aquatica</i>	48	85.7	12.42	Liu et al. (2013)
<i>Iris pseudacorus</i>		99	9.6	Wu et al. (2015)
<i>Phragmites australis</i>		64	10.51	Doherty et al. (2015a, 2015b)
<i>Typha latifolia</i>		91.2	93 mWm ⁻³	Oon et al. (2016)
<i>Elodea nuttallii</i>		97–98	184.75 mWm ⁻³	Oon et al. (2017)

highest power density of 18.1 mWm⁻² with 88% COD removal efficiency. In South Africa, Oodally et al. (2019) also investigated the performance of wetland plants in CW-MFC by comparing the performance of three indigenous South African wetland plants: *Cyperus proflifer*, *Wachendorfia thyrsoiflora*, and *Phragmites australis* with regard to removal efficiency and bioelectricity generation. From their study, the highest power density and voltage were obtained from the *Cyperus proflifer* plant species (229 ± 52 mW/m³; 510 mV). The removal efficiency of chemical oxygen demand was 97% ± 1% for *C. proflifer*, which was higher than *W. thyrsoiflora* (94% ± 1%), *P. australis* (94% ± 1%) and the control (unplanted) (90% ± 2%). In addition, the *C. proflifer* plant species achieved higher orthophosphate removal efficiency (98% ± 0%) than the control (72% ± 7%), *W. thyrsoiflora* (58% ± 6%) and *P. australis* (81% ± 4%). *C. proflifer* was the most suitable wetland in terms of electricity production and COD, ammonia and phosphate removal among the compared species (Oodally et al. 2019).

Despite the contribution of wetland plants to treatment efficiency and power generation, macrophytes, particularly emergent plants, have been identified as causing significant water loss in CW-MFCs through evapotranspiration. The treatment efficiency could be affected as the volume of wastewater flowing through the system declines due to water loss especially when the evapotranspiration rate exceeds 2.5 mm/d (Białowiec et al. 2014; Oon et al. 2017). Besides, in the absence of light, plant cell and microorganism respiration will take place and consume O₂. Hence, the dissolved oxygen (DO) level in the reactor reduces as the DO consumption exceeds production in the evening (absence of light). The plant's photosynthesis and respiration alter the oxygen dynamics of the reactor which ultimately will lead to voltage fluctuations (Doherty et al. 2015a, 2015b). Further

studies are required in these areas as far as the utilization of plant photosynthate in CW-MFC is a concern.

SUBSTRATE OR FILTRATION MEDIA

Substrates, also known as media, support matrix/material or filling material are another major component in CW-MFCs. Substrates play an integrated role in physical, chemical and more significant biological functions to eliminate (including filtering, trapping, adsorbing, biodegrading) the pollutants (Yang et al. 2018a, 2018b). They also serve as a support matrix for the living organisms and provide storage for many contaminants. In a CW-MFC, the preference of substrate or filtration media to be used is of major significance for the functioning of the entire system. On a practical level, cost and local availability are the two rudimentary factors determining the selection (Dordio & Carvalho 2013). More significantly, the physical (e.g., particle size, porosity, hydraulic and electrical conductivities, specific surface area, mechanical strength), chemical (e.g., surface charge, toxicity, and chemical stability), and biological (e.g., electron donors/acceptors) characteristics of the substrates must be considered for enhancing the system performance. For example, the sizes of the substrate particles have a determining effect on both the hydraulic characteristics and the porosity. Its permeability affects the wastewater flow in the system and it is where chemical and biological transformation by microorganisms occurs (Dordio & Carvalho 2013). Therefore, a very porous medium is often considered ideal, since it provides greater surface area for treatment contact and biofilm development. Several studies have employed the use of gravels, sand, compost, alum sludge and crushed marbles, both conventional and emerging substrates as

filtration media for CW-MFCs. However, in recent times, natural zeolite has often been preferred because of its crystalline and porous nature with larger specific surface area and adsorption ability. Natural zeolites are hydrated aluminosilicates of alkali and earth metals that possess infinite, strong, open, one- or three-dimensional crystal structures (Noori *et al.* 2006; Tuszyńska & Obarska-Pempkowiak 2008; Shuib & Baskaran 2011). They are good adsorbents of small molecules, have a high ability of riveting microorganisms, a higher removal efficiency of NH_3 and $\text{NH}_3\text{-N}$ from fluid solution and have a low cost which singles them out as a sustainable material for CW-MFC substrate material (Milán *et al.* 2008; Tuszyńska & Obarska-Pempkowiak 2008). An experiment conducted by Yakar *et al.* (2018) aimed at investigating the effect of various types of filtration media on the wastewater treatment process and bioelectric production in an up-flow constructed wetland coupled with a microbial fuel cell (UCW-MFC). The UCW-MFC system with zeolite (clinoptilolite) as filtration medium had higher treatment efficiency compared with other UCW-MFC systems with sand and volcanic cinder, with an average COD, NH_4^+ , NO_3^- and total phosphorus (TP) removal efficiency of 92.1%, 93.2%, 81.1% and 96.7%, respectively. On the other hand, the maximum power density was also recorded to be higher at 15.1 mW/m^2 compared with the other substrates used in the experiment. In another experiment conducted by Shuib & Baskaran (2011), comparing different substrates (zeolite, gravel and alum sludge), the use of zeolite as the substrate achieved a significantly higher COD and total nitrogen

(TN) removal with a four day HRT compared with the gravel and alum sludge substrates. Often, the use of fine conventional substrates like sand or clay with too small particle size (less than 0.2 mm) results in clogging (as shown in Figure 4), short-circuiting and lack of oxygen transfer.

Aside from the use of natural zeolite with high removal efficiency for NH_3 and $\text{NH}_3\text{-N}$, the use of alum sludge has widely been used in CW as a P-sorption substrate for the efficient removal of phosphorus (P) because of its chemical constituent (Al-richness). Further studies, however, are required in this area since different emerging substrates are specialized in certain targeted pollutants (Yang *et al.* 2018a, 2018b). In addition, exploring the use of more conductive materials like biochar, carbon stones, and charcoal needs to be assessed and the exploration of composite substrate materials can also be considered for optimum removal efficiency of all sources of contaminants since these materials similarly show the possibility of being used as filling materials.

MICROORGANISMS

The type of microorganisms that play an essential role in the performance of CW-MFCs is generally known as electroactive bacteria (EAB) or electrogens. These are bacteria with the ability to generate electrical energy through the oxidation of organic matter and transfer the generated electrons to an acceptor outside of their cells (Yadav *et al.* 2012; Guang *et al.* 2020). Electrogenic

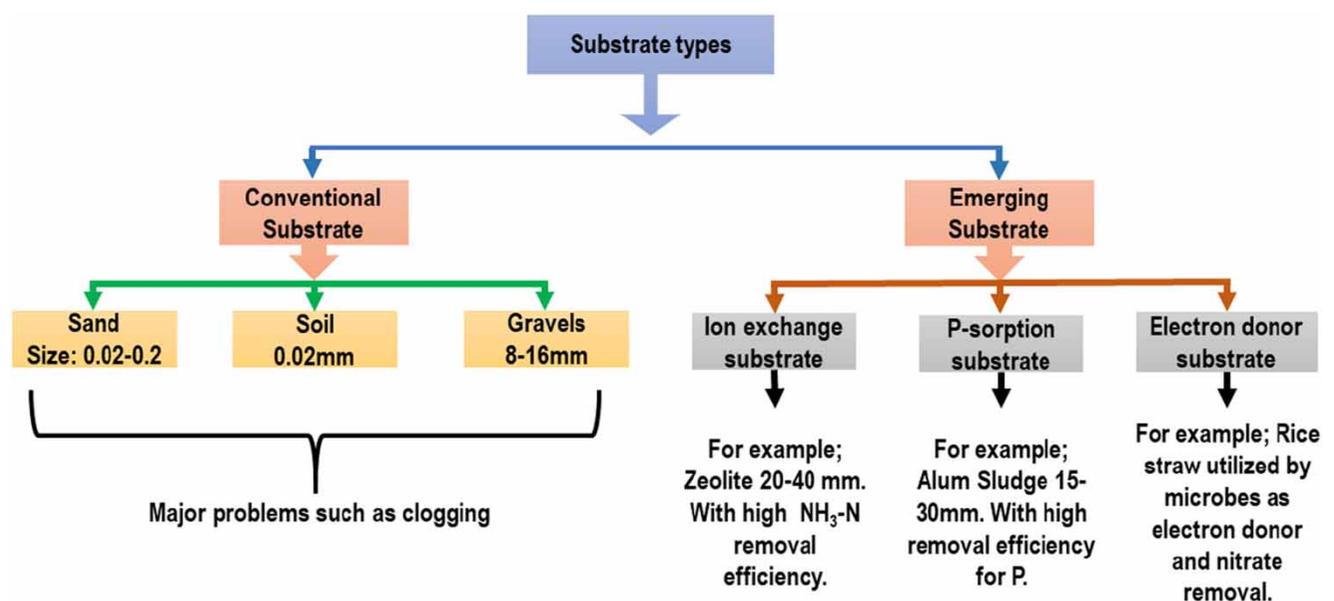


Figure 4 | Different types of substrates, their drawbacks and removal efficiency.

microorganisms are widely found in natural and polluted environments. Anaerobic sludge from industrial or domestic wastewater treatment plants, anaerobic sediment, primary industrial or municipal effluent and even farm soil are identified as germ sources for EABs (Shi *et al.* 2018; Guang *et al.* 2020).

In CW-MFC, the activities of EABs are one of the major determinants in the efficiency of the system. EABs catalyze substrate oxidation in the anodic compartment and simultaneously chemical and/or microbial substrate reduction reaction occurs in the cathodic compartment (see Figure 1). Anaerobic substrate oxidation by EABs produces carbon dioxide, protons, and electrons. The protons are transferred to the cathode and the electrons are transferred first to the anode and then flow to the cathode through an external circuit thereby producing electricity as the main product (Yadav *et al.* 2018). The metabolism of EABs, though important, is influenced by certain factors such as substrate composition, pH and temperature which favor predominant species in CW-MFCs and have been well documented (Srivastava *et al.* 2019; Guang *et al.* 2020).

ELECTRODES

As introduced earlier, electrodes are the main host for the redox reaction in the system and as a result, have a significant influence on the overall performance of the system. The choice of electrode material plays a pivotal role in CW-MFC (Shi *et al.* 2018). For a typical CW-MFC there are two kinds of electrodes: the anode electrode and the cathode electrode, both buried respectively in the anaerobic and aerobic regions of the reactor (Kalathil *et al.* 2018). By burying the anode and placing the cathode at the surface, CW-MFC can take advantage of the natural redox conditions approaching anaerobic at the anode, while the cathode is supplied with dissolved oxygen from the atmosphere and oxygen leakage from the rhizosphere of wetland plants (Kalathil *et al.* 2018). The optimization of this redox gradient between the anode and the cathode is very crucial in CW-MFC development for power generation (Doherty *et al.* 2015a, 2015b).

According to Doherty *et al.* (2015a, 2015b), the position of and the material used as the anode and the cathode is highly essential for the entire system performance, since it has a greater effect on the microbe-electrode interaction needed for biofilm development, substrate oxidation and electron transfer (Kalathil *et al.* 2018). Therefore, materials

with good electrical conductivity and low resistance, porosity, strong biocompatibility, chemical stability and non-corrosive, large surface area, good mechanical strength, that are easily made at low-cost, recyclable and scalable natural materials are considered ideal as electrodes (Kalathil *et al.* 2018; Shi *et al.* 2018).

Several electrode materials have been tried for their use and applicability as the anode and/or cathode in CW-MFCs. These include mainly carbon-based materials (e.g., graphite rod or plate, carbon cloth, carbon paper, carbon felt, granulated activated carbon), metal-based stainless steel and platinum as shown in Table 2. However, the carbon-based electrodes are widely used because of their ideal characteristics (Doherty *et al.* 2015c; Xie *et al.* 2015).

Aside from the appropriate selection of electrode material, another common bottleneck associated with CW-MFC electrodes is the electrode spacing. Electricity production to a large extent is also influenced by the electrode spacing (Doherty *et al.* 2015a, 2015b). To increase the availability of oxygen (O₂), cathodes are positioned near the air-water interface and rhizosphere zones (Yadav *et al.* 2012; Fang *et al.* 2013). However, because the anodes need to remain anoxic, they are buried deep from the surface of the CW-MFC, and this results in large electrode separation. Such separation increases the internal resistance of the system and thus lowers power output (Doherty *et al.* 2015c; Wang *et al.* 2017a, 2017b; Xu *et al.* 2018a, 2018b; Srivastava *et al.* 2019). In an experiment conducted by Wang *et al.* (2017a, 2017b) to assess the effect of electrode spacing on electricity production performance of CW-MFC, they considered 10 cm, 20 cm and 30 cm spacing between electrodes in three different reactors. Their results indicated that the maximum power density of CW-MFC was 2.55 W·m⁻³ when the electrode spacing was 10 cm, which was 30% and 50% higher than those with electrode spacings of 20 cm, and 30 cm respectively. The power density differences of CW-MFC with regard to different electrode spacing is, however, attributed to internal resistance. It is evident that the maximum power densities of CW-MFCs are lower mainly as a result of large ohmic losses (due to increment in internal resistance) and electrode potential losses (Wang *et al.* 2017a, 2017b). Therefore, for CW-MFCs to advance, it is important that the large internal resistances be combated. In view of this, aside from the need for appropriate selection of electrode material, the optimal spacing between anode and cathode electrodes is also very imperative. There is a need for further investigation in order to achieve the desired higher power density.

Table 2 | Summary of selected studies in CW-MFC using different substrates, macrophytes and electrode materials and their respective output efficiency (2012–2018)

Author	Substrate	Macrophyte	Electrode material	Initial COD (mg/L)	COD removal (%)	HRT (h)	Max. power
Yadav et al. (2012)	Gravel	<i>Canna indica</i>	Anode–Graphite Plate Cathode–Graphite Plate	1,500	74.9	96	15.7 mW/m ²
Zhao et al. (2013)	Gravel	<i>Phragmites australis</i>	Anode–Graphite Plate Cathode–Graphite Plate	1,058	76.5		9.4 mW/m ²
Fang et al. (2013)	–	<i>Ipomoea aquatica</i>	Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	180	86	72	0.302 W/m ³
Villaseñor et al. (2013)	–	<i>Phragmites australis</i>	Anode–Graphite Plate Cathode–Graphite Plate	250	80–100		0.15 mW/m ²
Liu et al. (2013)	–	<i>Ipomoea aquatica</i>	Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	193–205	94.8	48	12.42 mW/m ²
Fang (2015)	–	<i>Ipomoea aquatica</i>	Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	300	72.5	72	0.852 W/m ³
Doherty et al. (2015c)	–	<i>Phragmites australis</i>	Anode–Granular Graphite Cathode–Granular Graphite	411–854	64		0.268 W/m ³
Oon et al. (2015)	–	<i>Typha latifolia</i>	Anode–Carbon Felt Cathode–Carbon Felt	314.8	100		6.12 mW/m ²
Doherty et al. (2015c)	–	<i>Phragmites australis</i>	Anode–Granular Graphite Cathode–Granular Graphite	583	64		0.276 W/m ³
Srivastava et al. (2015)	–		Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	770–887	90.9		43.63 mW/m ³
Srivastava et al. (2015)	–		Anode–Granular Graphite Cathode–Granular Graphite	770–887	80.9		0.10 mW/m ³
Srivastava et al. (2015)	–		Anode–Granular Graphite Cathode–Pt-coated Carbon Cloth	770–887	84		320.8 mW/m ³
Srivastava et al. (2015)	–		Anode–Granular Activated Charcoal Cathode–Pt-coated Carbon Cloth	770–887	91.4		92.48 mW/m ³
Corbella et al. (2015)	Gravel	–	Anode–Cylindrical Graphite Rod Cathode–Cylindrical Graphite Rod	323	61	62.4	36 mW/m ²
Oon et al. (2016)	Gravel	<i>Taiifa latifolia</i>	Anode–Activated Carbon Cathode–Activated Carbon	624	99	24	93 mW/m ³
Xu et al. (2016)	DAS and PAC	NA	Anode–Powder Activated Carbon Cathode–Granular Graphite	500	80	60	87.79 mW/m ²
Corbella et al. (2016)	Gravel	<i>Phragmite australis</i>	Anode–Cylindrical Graphite Rod Cathode–Cylindrical Graphite Rod	323	60.6	62.4	131 mW/m ²
Xu et al. (2017)	DAS	NA	Anode–Granular gravel Cathode–Granular Activated Carbon	300	88.7	NA	2 W/m ³
Fang et al. (2017)	Gravel	NA	Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	300	91.05	72	0.38 W/m ³
Wu et al. (2017)	–		Anode–Graphite Plate Cathode–Graphite Plate	1,178	78		121 mW/m ²
Oon et al. (2017)	–	<i>Elodea nuttallii</i>	Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	643	97–98	24	184.75 mW/m ³
Srivastava et al. (2017)	Gravel	<i>Canna indica</i>	Anode–Graphite felt Cathode–Carbon Cloth		78.71	72	31.04 mW/m ³
Song et al. (2017)	Gravel	<i>Phragmites australis</i>	Anode–Granular Activated Carbon Cathode–Granular Activated Carbon	200	90.45	48	0.20 W/m ³
Xu et al. (2018a, 2018b)	Sand and ceramsite	<i>Phragmites australis</i>	Anode–Titanium mesh and Activated carbon Cathode–Titanium mesh		82	72	3,714 mW/m ²

HYDRAULIC RETENTION TIME

Hydraulic retention time, also known as hydraulic residence time (HRT), is a measure of the average length of time that a soluble compound remains in a constructed bioreactor. HRT can be expressed as:

$$\text{HRT} = V/(\theta * X).$$

where V = reactor volume, θ = the amount of feed inside the reactor and X = number of cycles per day; volume is in (m^3) and the influent flowrate is in (m^3/h) and HRT is usually expressed in hours (h) (Abdelgadir *et al.* 2014).

In the CW-MFC systems, HRT is the most influential factor that affects contact between substrates and microorganisms, which in effect favors higher treatment efficiency (Velvizhi 2019). When the hydraulic retention time is extended, the removal rate of the pollutants and the output power of the system can be improved, that is, the power generation performance of the system is enhanced. Yang (2015) studied the HRT performance of the system at 6, 12, 18, 24, 48 h, respectively. It is concluded that with the extension of HRT, the time for the CW-MFC system to reach a stable output voltage gradually increases, and the internal resistance gradually increases. When it becomes larger, the power density gradually decreases, and the Coulomb efficiency gradually increases. This is attributed to the fact that, with the extension of HRT, the matrix intercepts the organic matter in the sewage, the direct utilization of the dissolved organic matter in the sewage by the electrogenic bacteria, and the biodegradation of the organic matter trapped on the substrate by the microorganism, so that the Coulomb efficiency increases (Shi *et al.* 2018). Also in a study conducted by Fang *et al.* (2015), electricity production from Azo dye wastewater using a microbial fuel cell coupled constructed wetland operated under different operating conditions. The study assessed the effect of HRT on electricity production and the degradation characteristics of azo dye (ABRX3). A higher decolorization rate was achieved and electricity production reached a peak before slowing down with the elongation of HRT. However, the highest decolorization rate and electricity production were obtained when HRT was three days. At present, the recommended HRT of the CW-MFC system is generally two to three days. Enhancing HRT to achieve optimal performance of the system's power generation performance is one of the directions that need to be studied in the future (Shi *et al.* 2018).

FUTURE PERSPECTIVES AND CONCLUSION

From all earlier and recent studies on CW-MFC, there is no doubt that wetland plants, the substrate, the electrode material and the HRT play a significant role in the performance and efficiency of CW-MFC for wastewater treatment and electricity generation. However, most of the studies conducted specifically to assess the macrophyte effect on treatment efficiency were focused on nitrate, phosphorus and COD removal with less focus on heavy metal removal through the selection of appropriate macrophytes. In addition, wetland plants are recognized to play a crucial role in the performance and activities of electrogenic bacteria. However, each species used in CW-MFC has its chemical composition, ROL, ecological demands and physiological characteristics and these properties directly or indirectly affect removal efficiency as well as bioelectricity generation. Considering the vast array of wetland plant species and the minimal exploration in the suitable selection of macrophytes there is, therefore, the need for further study and exploration for wetland plants.

Also, CW-MFC studies carried out on substrate material and HRT effects are limited, including electrode materials. There is still room for further development of the CW-MFC electrodes as the material sciences are highly advanced. Highly porous and conductive materials could be explored. This technology has a great potential to be used in municipal wastewater treatment. Generally, removal efficiency in CW-MFC is appreciable in terms of some organic and inorganic contaminants, however, the aspect of energy generation needs great research attention to boost the energy generation capacity of the technology. Future studies could also focus more on finding suitable substrate and electrode materials to boost the scaled-up and industrial application of the integrated system.

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