

COMPARATIVE PERFORMANCE OF CARBOXYMETHYL CELLULOSE AS SUBSTRATE FOR ELECTRICITY GENERATION IN MICROBIAL FUEL CELL: A REVIEW

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Abstract— Due to the energy crisis globally and no proper utilization of renewable and non-renewable resources, different experimental design approaches and substrates have been employed to produce bioelectricity in an MFC. The major substrate tried to focus on in this review paper is carboxymethyl cellulose (CMC). Carboxymethyl cellulose is an important factor in Microbial fuel cell with great importance in the industry. No known enzyme is directly involved in the oxidation/reduction of CMC. However, carboxymethyl cellulases attack, specifically CMC. Distinct cellulose-degrading bacteria have been isolated for their higher cellulase activity. Similarly, pure bacterial cultures and co-cultures have been extensively used in degrading CMC for power and electricity generation. CMC concentration and the effect of different substitution factors also play an important role in voltage generation. This review gives an overview of CMC's current developments as substrate in MFCs and encourages developing more efficient processes for improved bioelectricity production in MFCs. It also discusses different ways to make enzymatic electrodes for current production using CMC fed reactor.

Keywords— CMC, microorganism, enzymatic electrode, cellulase enzyme, and microbial fuel cell (MFC)

I. INTRODUCTION

Complex biomass is the most abundant biopolymer substance in the world. However, the linkages between the monomers of the complex substrate make them hard to degrade into simpler carbohydrates. Limited study on the complex substrate in an MFC is mainly due to its low degradation rate and power output in MFCs (Ren *et al.*, 2007b, Rezaei *et al.*, 2008). Cellulose is an abundant carbohydrate source, and several attempts have been made to generate electricity in an MFC with cellulose as a sole electron donor. It is the polymer of glucose connected by β -2-4 linkages and is also associated with hemicellulose or lignin (Malherbe and Cloete, 2002). Several biological and chemical methods have been applied to convert cellulose into ethanol, H_2 , and methane, but there are economic and technical hurdles in these approaches (Howard *et al.*, 2003, Palmqvist and Hahn-Hägerdal, 2000). Carboxymethyl cellulose (CMC) is an essential derivative of cellulose with immense importance in the industry and daily life. It is a long linear chain of anionic

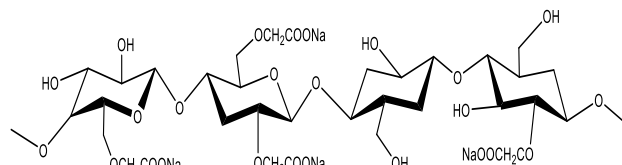


Figure 1: Carboxymethyl cellulose (CMC) molecular structure (Rani *et al.*, 2014)

polysaccharide from cellulose (Redman *et al.*, 2002), as illustrated in (Fig. 1)

CMC is an electrochemically inactive compound, and there is no known enzyme directly involved in catalyzing CMC's redox reaction. However, cellulase enzymes consist of exoglucanases, endoglucanases, and β -glucanases are used to hydrolyze CMC into glucose, cellobiose, and oligosaccharides. MFCs report the effectiveness of these enzymes. In the presence of electrochemically active microorganisms, it is unknown whether the cellulase enzymes are active in an MFC or hinder the power output.

II. HYDROLYSIS AND DEGRADATION OF CELLULOSE USING CELLULASES

Improving power generation using enzymes to increase enzymatic hydrolysis of cellulose is widely used. Enzymatic hydrolysis converts cellulose to sugar in bioethanol production (Sun and Cheng, 2002). Cellulases hydrolyze and degrade lignocellulosic and cellulosic waste discovered in 1983 from an anaerobic bacteria named *Clostridium thermocellum* (Khan *et al.*, 2010). Their production depends on the inoculum size, pH, temperature, aeration, medium, growth, and time (Immanuel *et al.*, 2006). Cellulase activity also depends on the presence of metal ions (Irfan *et al.*, 2012). Cellulases of bacteria and fungi have been widely classified into three classes that break cellulose into glucose (Bhalodia and Shukla, 2011). These enzymes include Endoglucanases (EC 3.2.1.4), Exoglucanases (EC3.2.1.91), and Carboxymethyl cellulases (β -glucosidases) (EC 3.2.1.21). This classification depends upon their ability to degrade carboxymethylated cellulose. Endoglucanases cleave the inner sites of cellulose to yield oligosaccharides whereas exoglucanases attack the reducing and non-reducing sites to liberate glucose and cellobiose (Ullah *et al.*, 2012), as shown (Fig. 2).

Cellulase enzymes have an essential role in industrial applications (Bhat, 2000). Many researchers are working to isolate microorganisms with higher cellulase activity (Ray *et al.*, 2007). Fungi and bacteria both produce

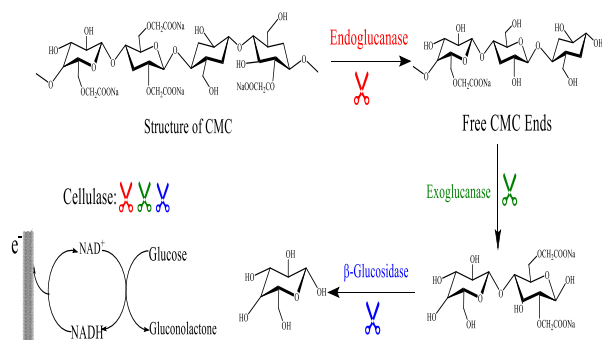


Figure 2: Schematic illustration of cellulase enzymes (endoglucanase, exoglucanase, β -glucosidase) to liberate glucose (Cheng *et al.*, 2012).

cellulase enzymes, but bacterial cellulase is better because bacteria grows faster than fungi and has higher yield of recombinant enzymes. Bacteria can also survive harsh environment during bioconversion process (Liu *et al.*, 2019). Cellulose degrading bacteria are isolated and characterized from industrial, municipal, agricultural, plant decayed, and organic matters but only a few of them are known to produce cellulose hydrolyzing compounds (Doi, 2008). Both aerobic and anaerobic microorganisms in different strategies to feed on cellulose are listed in Table 1. In abiotic conditions, 20% cellulose was hydrolyzed for 24h, and after 168h, it reached 51%. In the presence of bacteria, 50% of cellulose was degraded during 24h, which is 1.5 times higher than abiotic condition. After 168 h, the initial cellulose was degraded (60%) showing decrease rate of hydrolysis. Abiotic reactor showed higher enzymatic hydrolysis in MFCs due the reason of removing chemicals by microorganisms that contribute end product of enzyme inhibition. Enzyme + cellulose in MFC showed 74% cellulose degradation, while using only cellulose showed 15% (Rezaei *et al.*, 2008).

Many researchers have worked to isolate cellulolytic bacteria. In a study, among 32 strains isolated from MFCs using CMC agar medium for their cellulolytic activity, only ten showed their enzymatic activity for hydrolyzing cellulose as described in Table. 1 (Prabukumar *et al.*, 2015). Those bacteria included SAM3a (*Staphylococcus saprophyticus*), SAM212 (*Escherichia coli*), SAM4a (*Staphylococcus epidermidis*) SAM3b (*Enterobacter cancerogenus*), SAM1bigb (*Bacillus subtilis*), SAM4b (*Staphylococcus hominis*), SAM41 (*Staphylococcus haemolyticus*), SAM1a (*Lactobacillus murinus*), SAM213 (*Staphylococcus epidermidis*) and SAM223 (*Staphylococcus epidermidis*). These isolated strains showed distinct capabilities of hydrolyzing CMC and among these isolates *Staphylococcus saprophyticus* produced maximum enzymatic activity ($8.96 \pm 0.34 \text{ U mL}^{-1}$) followed by *Enterobacter cancerogenus* ($8.34 \pm 0.56 \text{ U mL}^{-1}$) and *Escherichia coli* ($8.17 \pm 0.11 \text{ U mL}^{-1}$) (Flimban *et al.*, 2019).

In another study, *Bacillus* sp., *Flavobacterium* sp., *Stenotrophomonas* spp and *Citrobacter* sp., were dominant in an MFC, and they had the potential to generate electricity (Chen *et al.*, 2017). Similarly, when *E. cloacae* bacteria was applied to produce electricity on different

carbon sources, maximum current density achieved from sucrose, glycerol, glucose, N-acetyl-D-glucosamine, cellulose, lactate and PB was $493 \pm 0.8 \text{ mA} \cdot \text{m}^{-2}$, $486 \pm 30 \text{ mA} \cdot \text{m}^{-2}$, $328 \pm 47 \text{ mA} \cdot \text{m}^{-2}$, $307 \pm 15 \text{ mA} \cdot \text{m}^{-2}$, $119 \pm 2.2 \text{ mA} \cdot \text{m}^{-2}$, $62 \pm 12 \text{ mA} \cdot \text{m}^{-2}$, and $18.5 \text{ mA} \cdot \text{m}^{-2}$ respectively (Rezaei *et al.*, 2009). Another bacterial specie (KW16) belonging to *Bacillus* produced a maximum electric potential of $81.9 \pm 0.25 \text{ mV}$ in MFC operation. Its cellulase activity was $6.84 \pm 0.3 \text{ IU mL}^{-1}$ in the presence of 0.5% CMC as a carbon source (Basene and Gonthalwal, 2019). This isolated strain KW16 contained a powerful enzyme oxidase or dehydrogenase, which helped in electricity generation.

III. DISTINCT MICROORGANISMS USING CELLULOSE AND CMC FOR ELECTRICITY GENERATION

Microbial Fuel Cell (MFC) is a promising technology for wastewater treatment and power generation for biodegradable organic waste (Chauhan, 2013). It uses electrochemically active microorganism as biocatalyst. Few researchers have worked on cellulosic waste because no known microorganism can hydrolyze cellulose and transfer electrons to extracellular substrates. Conversion of cellulosic waste to electricity required a syntrophic consortium that uses both electron donor (cellulose) and electron acceptor (anode), producing unique pressure on the microbial community (Ren *et al.*, 2007a). In electricity production from cellulosic biomass in MFCs, the ideal exoelectrogen must hydrolyze cellulose and utilize anode as electron acceptor without using redox mediator for mass transfer of electron to modified electrode because they are toxic and have replenished subsequently (Rismani-Yazdi *et al.*, 2007).

A known co-culture *Clostridium cellulolyticum* and exoelectrogen *Geobacter sulfurreducens* have been used successfully to convert cellulose into electricity in MFCs, as listed in Table 2. The binary culture of *G. sulfurreducens* and *C. cellulolyticum* converted cellulose to electricity without using enzymatic pretreatment or catalysts. *C. cellulolyticum* converted cellulose into acetate, hydrogen, carbon dioxide, and ethanol in this system, while *G. sulfurreducens* transferred electron from these fermented products to the anode by anaerobic respiration. No known bacterial strains performed the complete conversion. Therefore combining this cellulolytic and exoelectrogenic activity is needed for current production. It was proposed through this fermentation that anaerobic respiration has an advantage over anaerobic oxidation based on the energy per electron transfer (Lovley, 2006, McInerney and Beaty, 1988). The maximum power density ($143 \text{ mW} \cdot \text{m}^{-2}$) by co-culture using CMC was compared to the power output (154 mW/m^2) of *G. sulfurreducens* using acetate as an electron donor. This data showed higher results than two chamber of MFCs system (75 mW/m^2), where 20 mM was used as mixed cultures and ferricyanide as catholyte (Oh *et al.*, 2004).

Compared to the mixed cultures in MFCs, pure cultures have also been used as a source of electricity generation (Hassan *et al.*, 2012). When cellulose was used as

Table 1. List of Cellulolytic Bacteria

Aerobic	<i>Acinetobacter junii</i> , <i>Acidothermus cellulolyticus</i> , <i>Anoxybacillus</i> sp., <i>Bacillus subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> , <i>B. amyloliquefaciens</i> , <i>B. circulans</i> , <i>B. flexus</i> , <i>Bacteroides</i> sp., <i>Cellulomonas biazotea</i> , <i>Cellvibrio gilvus</i> , <i>Eubacterium cellulosolvens</i> , <i>Geobacillus</i> sp., <i>Microbispora bispore</i> , <i>Paenibacillus curdlanolyticus</i> , <i>Pseudomonas cellulose</i> , <i>Salinivibrio</i> sp., <i>Rhodothermus marinus</i> .
Anaerobic	<i>Acetovibrio cellulolyticus</i> , <i>Butyrivibrio fibrisolvens</i> , <i>Clostridium thermocellum</i> , <i>C. cellulolyticum</i> , <i>C. acetobutylicum</i> , <i>C. papyrosolvens</i> , <i>Fibrobacter succinogenes</i> , <i>Ruminococcus albus</i> .

substrate in MFC using the pure bacterial cultures, no electricity was produced using CMC as a substrate, and the bacterial strains produced a voltage of 365 ± 5 mV. Similarly, Rumen bacteria are the well-known microorganisms for cellulolytic activity under an aerobic situation, although their electrochemically activity has not been previously reported. The data showed that cow rumen bacteria degraded cellulose during MFC experimental conditions. The power density was 55 mWm^{-2} by using rumen bacteria as biocatalyst and cellulose as substrate (RismaniYazdi *et al.*, 2007). This power output is low compared to the modified electrodes or designed compartments (Gil *et al.*, 2003) with power generated under similar operating conditions from organic and soluble in two-chamber MFCs system (Phung *et al.*, 2004). It is also reported that *E. cloacae* can be used as a sole source for cellulose degradation (Rezaei *et al.*, 2009). *E. cloacae* has 1,4- β -D-glucanase activity and thus degrade cellulose (Sami *et al.*, 2008). A new electrogenic strain *Dietzia* sp. RNV-4 bacterium has been isolated and identified as electrochemically active (Sacco *et al.*, 2017). *Dietzia* sp. RNV-4 bacterium, when used in Microbial fuel cell produced current.

IV. COMPARATIVE PERFORMANCE OF MFCs USING CMC AND OTHER DISTINCT SUBSTRATES

Distinct researchers have worked on using cellulose and CMC as a source of electricity generation in MFCs. It was observed that the efficiency of the biofuel cell (BFC) with CMC substrate was higher than other cellulose biomass, as summarized in Table 3. The conversion technology of CMC could be improved if an enzymatic catalytic system is combined with biofuel cell, which provides a

Table 2. Microorganisms employed for bioelectricity generation in MFC using cellulose and CMC as carbon source

Microorganisms	Carbon Source	Power Density mW/m^2	References
Nocardiosis sp. KNU (S. Strain)	CMC	162	(Hassan <i>et al.</i> , 2012)
Streptomyces enissocaesilis KNU (K. strain)	CMC	145	(Hassan <i>et al.</i> , 2012)
Co-culture (<i>Clostridium cellulolyticum</i> and <i>Geobacter sulfurreducens</i>)	CMC	143	(Oh <i>et al.</i> , 2004)
<i>Enterobacter cloacae</i>	Cellulose	4.9	(Rezaei <i>et al.</i> , 2009)
Rumen from men of Cow	Cellulose	55	(Rismani-Yazdi <i>et al.</i> , 2007)

better opportunity in a simple way for direct conversion of chemical energy stored in CMC into electrical power. When an anodic chamber of MFCs was filled with 0.5% and 1% CMC as substrate inoculated with bacterium *Exiguobacterium* sp SU-5, the current production was stable to 1067mV at 18thh. It was comparatively higher in Nafion membrane-fitted MFCs than salt bridged MFCs nearly 500mV at 94h due to the minimal transfer of an electron from anodic to the cathodic chamber in salt bridge than Nafion membrane. However, 1% CMC concentration with Exiguo bacterium sp SU-5 produced electricity production in the range of 1350-1400 mV in Nafion fitted MFC at 20h. In contrast, the salt bridge system had nearly 500 mV of electricity generation at 168h (Nagesh *et al.*, 2019).

It has also been reported that CMC concentration is an essential factor for voltage generation. CMC as a carbon source is commonly used in the anode. Voltage was monitored with CMC concentrations ranging from $1 \text{ g} \cdot \text{L}^{-1}$ to $50 \text{ g} \cdot \text{L}^{-1}$. At $25 \text{ g} \cdot \text{L}^{-1}$ of CMC concentration, the maximum output voltage was achieved from 413mV to 1075 mV, which decreased when the CMC concentration was further increased from $25 \text{ g} \cdot \text{L}^{-1}$ (Wang *et al.*, 2014).

V. CMC DEGRADATION AND ITS COLUMBIC EFFICIENCY

Various MFC conditions were compared for the CMC and other substrates degradation in Fig. 3. For the 1 gL^{-1} CMC test, $42 \pm 5\%$ sugar in CMC was used by *C. cellulolyticum* system. The co-culture with CMC was hydrolyzed in 16 days up to $64 \pm 4\%$, which was 52% higher than pure culture, while another co-culture degraded

Table 3. Electricity generation using cellulose and CMC in Microbial fuel cells

Energy sources	Energy conversion technology	Open circuit potential ^a (V)	Maximum power density (W cm^{-2})	Ref.
Carboxymethyl cellulose	Microbial fuel cell	0.8	14.3	(Ren <i>et al.</i> , 2007b)
Cellulose	Microbial fuel cell	0.8	5.9	(Ren <i>et al.</i> , 2007b)
Cellulose	Microbial fuel cell	ca. 0.5	ca. 8	(Rezaei <i>et al.</i> , 2007)
Cellulose	Microbial fuel cell	ca. 0.8	ca. 10	(Rezaei <i>et al.</i> , 2008)
Cellulose	Fuel cell	ca. 0.44	4.4	(Sugano <i>et al.</i> , 2010)
Cellulose sulfate	Biophotofuel cell	0.56	ca. 30 ^b	(Kaneko <i>et al.</i> , 2006)
Carboxymethyl cellulose	Biofuel cell	0.75	128	(Cheng <i>et al.</i> , 2012)

^aPotential vs. Ag/AgCl.

^bCalculated from its FF value when operating in ambient air atmosphere.

Table 4. Comparative performance of MFCs with other distinct substrates

Substrate	Reactor type ^a	Maximum voltage (mV)	Maximum power (mW/ m ²) ^b	Duration of current production	Reference
Glucose + powdered corn stover	AC	380	331 (800 X)	14	(Wang <i>et al.</i> , 2009)
Glucose + residuals from steam exploded corn stover	AC	410	406 (600 X)	12.5	
Powdered corn stover	AC	86	Not available (NA)	NA	(Zuo <i>et al.</i> , 2006)
Corn stover hydrolysate (from steam explosion)	AC	500	952 (250 X)	1	
Microcrystalline cellulose	TCFC	475	55	5.2	(Rismani-Yazdi <i>et al.</i> , 2007)
Soluble carboxymethyl cellulose	TCFC	800 (50 kX)	143 (770 X)	24	(Ren <i>et al.</i> , 2007a)
Amorphous + microcrystalline cellulose	TCFC	800 (50 kX)	59.2 (890 X)	24	
Microcrystalline cellulose + cellulose enzyme	TCAC	700	98 (1 kX)	30	(Rezaei <i>et al.</i> , 2008)
Microcrystalline cellulose	TCFC	8	18.2 (5 kX)	10	(Rezaei <i>et al.</i> , 2009)
Corn cob	Tubular AC	672	7.18 (50 X) ^c	>67	
					(Gregoire and Becker, 2012)

^aAC, single chamber air cathode MFC; TCFC, two-chamber MFC with ferricyanide in cathode compartment; TCAC, two-chamber MFC with air-sparged cathode compartment.

^bResistance at which the maximum power was measured is given in parentheses, if available.

^cPower density was calculated based on the cathode surface area (0.152 m²), which was the same in the two trials

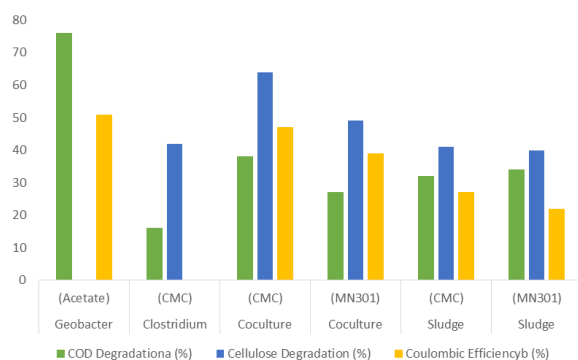


Figure 3: Summary of substrate degradation and electron recovery.

CMC with MN301 was reached to stable point $49 \pm 6\%$ was less than CMC but comparatively greater than CMC fed *C. cellulolyticum* pure culture. The sludge inoculum for CMC and MN301 showed a similar amount of degradation $41 \pm 5\%$ and $40 \pm 7\%$, respectively (Ren *et al.*, 2007a).

VI. CMC FED REACTOR

There are different ways to make enzymatic electrode for current production using CMC fed reactor. Glassy carbon (GC) electrodes (geometric surface area of 3mm), used as a substrate to generate electricity. CVs (Cyclic voltammograms) of GDH modified anode consist of 0.1M acetate buffer (pH 5.5), adding cellulase enzyme (0.5mg/mL), 1% CMC and 10mM NAD⁺. CV was run immediately after adding cellulase enzyme. This result suggested glucose production from CMC by the serial catalyst of cellulase enzyme and glucose catalytically oxidation with NAD⁺ and GDH as a cofactor (Cheng *et al.*, 2012) is described in (Fig. 4).

VII. CMC AND MN301 FED REACTOR

In CMC fed reactor, *C. cellulolyticum* was found in suspension with *Geobacter* cells with comparatively low cell density. In MN301 fed reactor *G. sulfurreducens*

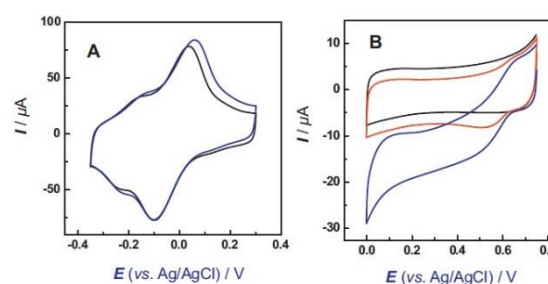


Figure 4: CVs of GDH bioanode with 0.1M acetate buffer (pH 5.5) and 1% CMC.

were seen in suspension while *Clostridium* cell was attached to cellulose particle. It was observed that both *Clostridium* and *Geobacter* stayed on biofilm and formed aggregate instead of the anode's surface. Fish results measured *C. cellulolyticum* and *G. sulfurreducens* abundance on real PCR analysis separately. Based on the given results, *Clostridium* suspension was greater than *Geobacter* for about 21 and 370 times in both substrate CMC and MN301, respectively. *Geobacter* cell was visualized in MN301 suspension sample, in addition, there was 5 thousand times higher *Clostridium* density in CMC biofilm than MN301, and 25 times more *Geobacter* were seen attached on the anode in MN301 reactor than CMC reactor as shown in (Fig. 5a and b).

VIII. CONCLUSIONS

In the past years, glucose and acetate were used as simple substrates. Still, nowadays, researchers are trying to use more unconventional substrates of waste biomass utilization and improvement of MFCs output performance. Substrates in the form of both complexity and strength are being used in MFCs. A simple substrate was easily degraded and improved the hydrogen and electric output of the system. In contrast, a variety of exoelectrogens were formed when the complex substrate was used in the MFCs system. Substrates such as carboxymethyl cellulose and starch performed better in comparison to all

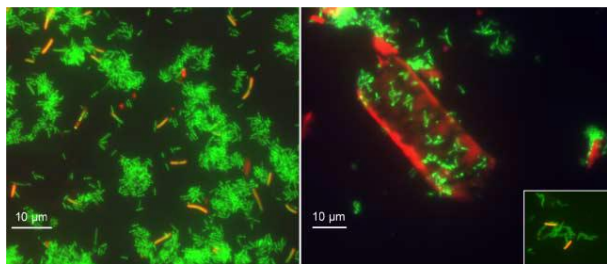


Figure 5: (a) CMC-fed MFC (b) MN301-fed MFC. Cellulose particles were stained with Congo red in MN301.

other polysaccharides studied to date. The MFCs performance with starch substrate attributed to being faster hydrolysis rates as compared with cellulose and chitin.

IX. LIMITATIONS AND FUTURE PROSPECTS

Complex substrates were compared with CMC as a carbon source for electricity production in this review. In addition, the enzymatic electrodes, including CMC fed reactors, have also been reviewed. Several studies have been carried out on anodes and cathodes in MFC, while fewer studies have seemed on the utilization of complex substrate and pure cultures of microorganisms for bioelectricity generation. Although many exoelectrogenic bacteria have been identified but few of them are known to hydrolyze cellulose by using cellulases (specifically carboxymethyl cellulases) for power and electricity generation. In addition, different strategies could be applied for the utilization of complex biomass in MFC, three of which are: a) direct conversion of solid particulate biomass in a solid substrate MFCs, b) hydrolysis of solid biomass in a liquid MFC, and c) hydrolysis of externally particulate solid substance, followed by the extraction and conversion of these extracted compounds in a liquid MFC. Moreover, when it comes to the usage of the non-soluble substance, the size of the substrate might also play an essential role in power generation.

The critical points for future aspects are given below:

1. To improve the output of electric power and electric current, more technological advancements are needed in terms of material, costs and substrates at a level where they can be commercially exploited.
2. Several new substrates should be exploited under the MFC setups. These may include the wastewaters from molasses based on distilleries rich in organic matter, wastewater from biorefineries, wastewaters from the pharmaceutical industry with recalcitrant pollutants, and plant biomass waste (agriculture residue).

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflicts of interests.

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