



Simultaneous Investigation of Three Effective Parameters of Substrate, Microorganism Type and Reactor Design on Power Generation in a Dual-Chamber Microbial Fuel Cells

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Background: The use of Microbial Fuel Cells (MFCs) has been expanded in recent years due to their ability in producing bioelectricity and treating wastewater simultaneously. However, there are still some obstacles to use MFC on an industrial scale. Regardless of the restriction of electrodes applied in the electron transferring process, there are also some other factors having strong roles in reducing the power density of MFCs.

Objectives: In this paper, the effect of three categories of limiting factors such as kinds of microorganisms (*Saccharomyces cerevisiae* and *Shewanella* sp.), substrate type (Glucose and acetate), and features reactor components have been investigated on the power density generation. Simultaneous investigation of these parameters and demonstration of which parameters would induce more power density can help to improve the scale-up of MFCs.

Materials and Methods: Two types of MFCs with different designs were constructed and inoculated with pure cultures of *Saccharomyces cerevisiae* PTCC 5269 and *Shewanella* sp. The OCV (Open Circuit Voltage) and polarization curves of MFCs were measured when the quasi-steady-state condition was observed.

Results: Based on results, utilizing acetate in the presence of both microorganisms led to approximately 60% higher power density compared to glucose. The comparison of maximum power densities of different reactor designs indicated an approximately 17-70 % increase of power generation. However, the resultant shows modification of reactor design even when other parameters are not optimal can increase power density more than three times.

Conclusion: Actually, reactor design has the most important role in the power density with the MFC while the effects of substrate and microorganism parameters are not inappreciable.

Key Words: Dual-chamber microbial fuel cell, Microorganism, Power density, Reactor components, Substrate

1. Background

Microbial fuel cells (MFCs) are noticeable devices that convert various wastes directly into electricity using electrogenic microorganisms. Actually, MFCs are capable of producing renewable energy and performing wastewater treatment simultaneously (1, 2). A customary laboratory MFC is consisted of anodic and cathodic chambers in which microorganisms oxidize the substrate and produce electrons and protons in the anode chamber. Electrons are transferred to cathode by external circuit and protons are transported through the cation exchange membrane internally. The MFC is influenced by several critical factors divided in to three parts: Microbiology effects such as, ideal microorganisms or a consortium of microorganisms (3),

metabolism of microorganisms and electron transferring pathways toward electrodes (4). The second involves structural factors such as, the cell design (5), type of electrode materials and modifying electrode surfaces (6, 7), the distance between the electrodes (8) and the last part is operating factors such as, materials and solution chemistry (9), solution pH (2), wastewater alkalinity, buffers and their concentration, ionic strength, solution conductivity (10, 11), temperature (1), operation mode in relations to fed-batch or continuous flow (12, 13) and different types of wastewater (14, 15).

In this study, three major parameters (substrate, microorganism, reactor design) in power generation have been investigated, simultaneously. Finally, it should be determined which parameters have greater

impact on the power density of MFC.

MFCs are being operated using any form of biodegradable organic matter as a substrate, including glucose, acetate, monosaccharides and complex carbohydrates such as starch, fatty acids, amino acids and proteins, biodegradable organics in food wastewater, swine, human wastewater and domestic wastewater (16). Kinds of substrate also usually affect the MFC performance from two aspects i.e. affecting the composition of the microbial community in the anode biofilm formation which consequently affects the coulombic efficiency and MFC power density through the amount of electron production. Glucose is considered as a substrate with a relatively lower coulombic efficiency due to diverse competing metabolisms such as fermentation and methanogenesis compared to lower molecular compounds, such as acetate (16). On the other hand, acetate is a simple carbon source that its inertness against fermentations and methanogenesis metabolisms caused inadequate growth of various kind of bacteria (16). Glucose and acetate participate in the following reactions in MFC.

Acetate reaction: $C_2H_3O_2^- + 4H_2O \rightarrow 2HCO_3^{2-} + 9H^+ + 8e^-$

Glucose reaction: $C_6H_{12}O_6 + H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$

However, electron transferring through the biofilm in anode is another critical factor enhancing the MFC performance. *Saccharomyces cerevisiae* as a eukaryotic microorganism is a very attractive organism to work with since it is nonpathogenic. Susceptibility to genetic modifications by recombinant DNA technology with *S. cerevisiae* make this organism attractive for several biotechnological purposes. Electron transferring of *S. cerevisiae* is occurred directly to the anode (17). The metal-reducing bacteria strains such as *Shewanella* sp. are able to transfer electrons through three different pathways as the direct electron transfer which is an extracellular electron transfer without any electron mediators, through conducting pili and excreting quinone-like molecule such as flavins that mediate extracellular electron transfer. *Shewanella* is able to utilize many carbon sources and can be used to generate electricity from many substrates under anaerobic conditions. (18, 19).

MFCs are being constructed using a variety of materials and diversity of configurations. MFC reactor design is one of the crucial factors which directly influence on the performance of MFCs. Key issues dealing with MFC configurations such as spacing, shape and orientation, size of electrodes and effective electrodes surface area will ultimately determine the maximum power attainable for a given design (20, 21).

Although, there are several studies investigating the effect of above mentioned individual parameters on the

performance of MFC (22, 23), simultaneous investigation of the effects of microorganism type, kind of substrate and reactor type has not been conducted in one study. In this study, two MFC reactor design, two substrates and two microorganism types have been chosen so that each of them can make a significant difference in the power production in MFC. The highest power densities have all been obtained under nearly ideal conditions of reactor design, substrate and microorganism type. However, this issue that which parameter(s) is more effective in power generating is still lacking. It should be noted that the two-reactor design used in this investigation are different in electrodes and membrane surface area and in the other conditions such as volume, shape and orientation are the same. The two different reactors used was designed to incorporate different membrane and electrode surfaces and investigate their importance in affecting the power generation (24, 25).

2. Objectives

In this research work, the key factors will be investigated together in order to find out the most important variable producing the maximum power density. The results of this simultaneous investigation will help future development of MFCs.

3. Materials and Methods

3.1. Growth Condition of Microbial Strain

A pure culture of *Saccharomyces cerevisiae* PTCC 5269 was procured from Iranian Research Organization for Science and Technology (IROST), Iran, Tehran, and pure culture of *Shewanella* sp. IBRC-M 4029 was procured from Iranian Biological Resource Center, Iran, Tehran. The microbial cells were grown in a sealed bottle with agitation of 160 r/min and temperature of 27 °C and 34 °C for *S. cerevisiae* and *Shewanella*, respectively. The media and growth condition of each microbial strain was different and was according to **Table 1**.

All chemicals and reagents used in this study were of analytical grade and were supplied by Merck (Germany). The media was sterilized and autoclaved at 121 °C and 1 atm for 15 min. The media pH was initially adjusted to 7 and 8 for *S. cerevisiae* and *Shewanella*, respectively. The pH meter, Crison Basic 20⁺ (Spain) model glass-electrode was employed for measuring the pH values of the aqueous phase. The initial pH of the working solutions was adjusted by adding diluted 0.1 M solutions of HCl or NaOH when required. The growth of each microbial strain was monitored by optical density measurement (OD) using spectrophotometer (T80⁺, PG Instrument Ltd., England) at wavelength of 660 nm.

Table 1. Media Composition and Culture Condition for *S. cerevisiae** and *Shewanella**

Microorganism	<i>S. cerevisiae</i>				<i>Shewanella</i>			
	Sample 1		Sample 2		Sample 1		Sample 2	
Medium component (g l ⁻¹)	Glucose	2.5	Acetate	2.5	Glucose	0.5	Acetate	0.5
	Yeast extract	2.5	Yeast extract	2.5	Yeast extract	10.0	Yeast extract	10.0
	Peptone	3.0	Peptone	3.0	Peptone	2.5	Peptone	2.5
	Glucose	2.5	Glucose	2.5	Glucose	0.5	Glucose	0.5
	NH ₄ Cl	1.0	NH ₄ Cl	1.0	NaCl	20.25	NaCl	20.25
	KH ₂ PO ₄	0.5	KH ₂ PO ₄	0.5	MgCl ₂	1.75	MgCl ₂	1.75
	K ₂ HPO ₄	0.7	K ₂ HPO ₄	0.7	MgSO ₄	2.4	MgSO ₄	2.4
					KCl	0.5	KCl	0.5
					CuCl ₂	0.09	CuCl ₂	0.09
					NaBr	0.0065	NaBr	0.0065
Culture condition								
Temp** (°C)		27	27			34		34
pH		7.0	7.0			8.0		8.0
r/min		160	160			160		160
Volume (mL)		100	100			100		100

* After 24-48 h cultivation of the 100 mL above culture, another 400 mL of each media was also prepared and poured to the MFC anode chamber in an aerobic condition and temperature 28±2 °C for further investigation.

** Temp: temperature

3.2. MFCs Construction

Two types of MFCs with different designs were constructed as shown in **Figure 1**. Both anodic and cathodic chambers of fabricated MFC₁ and MFC₂ in the laboratory scale were made of plexiglass. The volume of each chamber in MFC₁ and MFC₂ (anode and cathode chambers) was 600 mL and the related working volume was 500 mL. Nafion 117 (DuPont, Wilmington, USA) was used to separate anode and cathode

compartment. Carbon cloth was used as electrode for both MFCs (plain, T-300, Toray) and projected surfaces of electrodes and membrane were 60 cm² and 32 cm² for MFC₁ and MFC₂, respectively. Prior to use, the electrodes were immersed in distilled water for an hour and membrane was under acid and hydrogen peroxide treatment. The cathode and anode were connected with titanium wire and voltage was recorded using a digital multimeter (Fluke 289 True RMS).

(A)

(B)



Figure 1. MFC equipments; MFC₁ (A) and MFC₂ (B)

3.3. Operating Procedures

Before running the MFCs, growth curves of microbial strains by optical density and dry mass methods were investigated and the optimum condition for presentation

of microorganisms in MFCs was obtained (26). The microorganisms were read at 660 nm for optical density on T80+-PG UV visible spectrophotometer, 2 times each dilution. Dry mass method just was applied for

certainty of accuracy of growth curves obtained by OD method. The microorganisms in 10 mL medium were centrifuged for 10 min, washed with sterile double distilled water, dried for 18 h and weighed.

Phosphate buffer with pH = 6.8 was used as a catholyte and in order to aerate the cathode, air pump was used. MFCs were sterilized and operated for 7 days in the batch mode and then polarization curves were measured using various external resistances.

3.4. Data Acquisition and Measurements

The OCV (Open Circuit Voltage) of MFCs was measured by leaving the circuit in an open mode until reaching a steadiness in respect to voltage. Polarization curves were obtained by varying the external resistance in the range

of 1,000,000-33 Ω . The data was taken only when quasi steady-state condition was observed (20 min after changing the external load). The current (I, A) was calculated by $I = E/R$ where E is voltage (V) and R is resistance (Ω). The power output of the cells (P, W) was calculated as $P = IV$. Power and current were normalized by using projected surface area of the cathode and anode (27, 28).

4. Results

4.1. The Interdependent Effect of Substrates and Microbial Diversity on the Power Generation of MFC

The growth curves of *S. cerevisiae* and *Shewanella* sp. are represented in **Figure 2**.

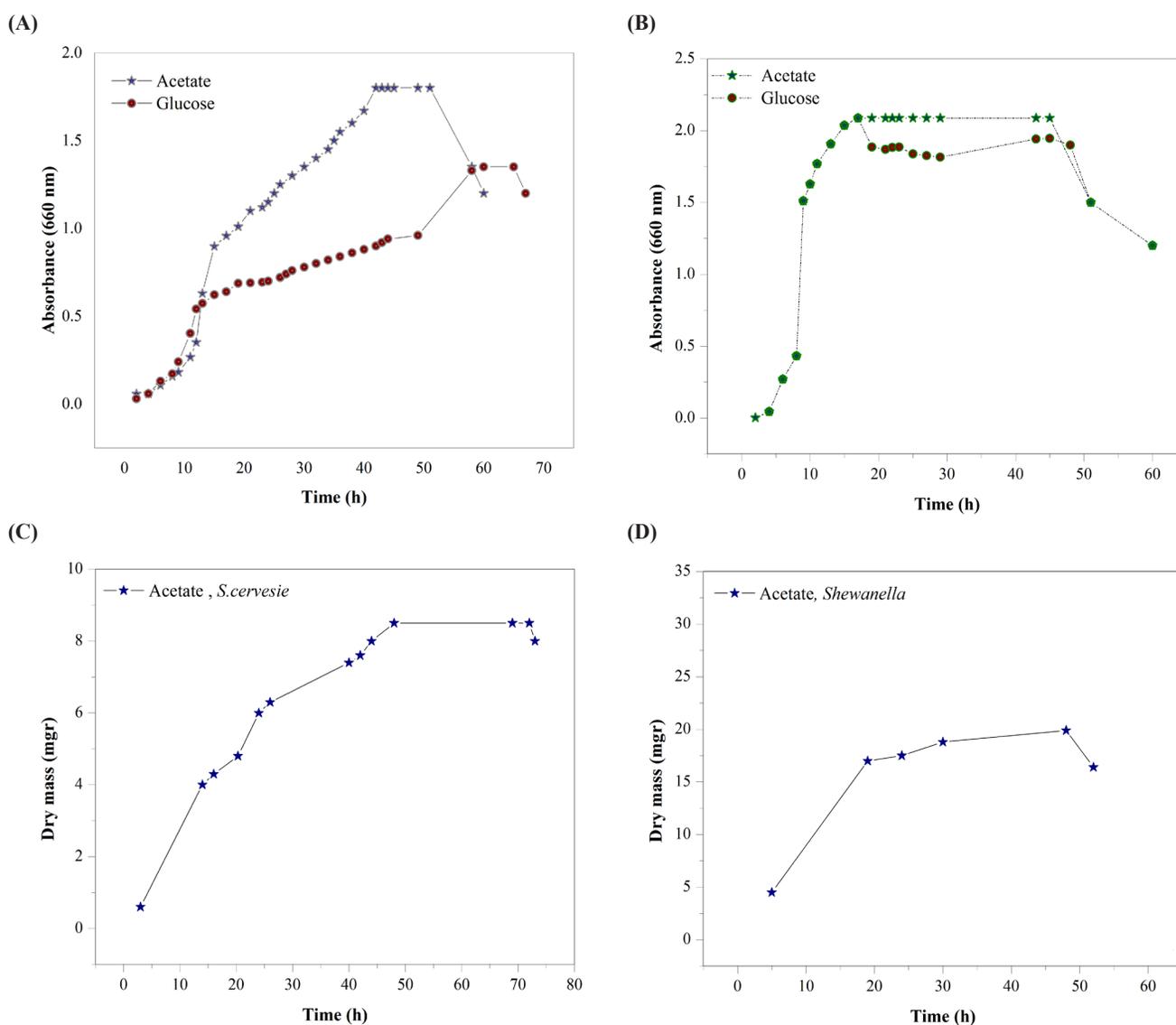


Figure 2. (A) Growth curves of *S. cerevisiae* in an aerobic condition in presence and absence of acetate by optical density method. (B) Growth curves of *Shewanella* sp. in an aerobic condition in presence and absence of acetate by optical density method. (C) Growth curve of *S. cerevisiae* in an aerobic condition in presence of acetate by dry mass method. (D) Growth curve of *Shewanella* sp. in an aerobic condition in presence of acetate by dry mass method

The pattern obtained from the growth curve of *S. cerevisiae* showed that the microbial population was stable for 42-51 h in the medium containing acetate and stability of the medium without acetate was in the range of 58-65 h. The results have shown that the microbial population of the media containing acetate, in the presence of both microorganisms is about 1.4 more than that of the medium without acetate. This is probably due to biosynthetic pathway and flux distributions in central metabolism of both microorganisms (29). In order to maintain and keep the growth steady, most of microorganisms should possess a kind of switching between physiological programs of fast growing in the presence of abundant nutrients to the one in the absence of nutrients. An example of such program, is called “acetate switch” (30). The case in which acetate is incorporated to the medium in comparison to the situation where glucose exists in the medium, during exponential growth, cells consume glucose and dissimilate acetate. By diminishing the glucose, the process of acetate assimilation starts and leads to more growth (more cells). The above results has not only been observed by non mixotrophic microorganisms to produce more cells, but has also been observed by mixotrophic microorganisms and microalgae (31). On the other hand, acetate is produced by *S. cerevisiae* and *Shewanella* when growing on medium containing glucose as central metabolism. Actually, this production is directly proportional to the glucose consumption (about 2.3 mg of acetate/g glucose) (32, 33). So it can be concluded that the type of substrate and microbial diversity in MFCs are very interdependent (34). On average, the maximum power density was varied with the substrate types as shown in **Figure 3a**.

4.2. The Effect of Reactor Design on the Power Generation of MFC

The acetate-fed-MFCs showed the higher power density in the presence of both *S. cerevisiae* and *Shewanella* compared to glucose-fed-MFCs. These outcomes were confirmed in both MFC₁ and MFC₂ (as shown in **Figure 3**) and they are also consistent with previous results in which acetate was the preferred substrate for electricity generation in MFC (23, 35). The maximum power density generated by acetate in the presence of *S. cerevisiae* was 2100.8 μWm^{-2} and in the presence of *Shewanella* it was as 4104.1 μWm^{-2} . The maximum power density generated by glucose in the presence of *S. cerevisiae* is 47.8% lower than that of produced with acetate. Also, the maximum power density generated by glucose in presence of *Shewanella* is 38% lower than that of produced with acetate. The lower

maximum power density of MFC with *S. cerevisiae* compared to the *Shewanella* was due to their different electron transfer mechanisms. In *Shewanella* electron transferring operated by three pathways, although there is no way except direct electron transfer in *S. cerevisiae* (**Fig. 3b**) (18, 19).

In general, according to the results, acetate yielded a higher current density which was approximately 2-3 times more than that of the measured in the presence of glucose (35.8 versus 13.3 mA m⁻² and 39.6 versus 19.8 mA m⁻², in the presence of *S. cerevisiae* and *Shewanella*, respectively), indicating faster bacterial uptake of acetate in comparison to glucose (32). On the other hand, with respect to the different electron transfer pathway of *S. cerevisiae* and *Shewanella*, the obtained power density in presence of *Shewanella* is quite in accordance with the expectation.

The performances of MFC₁ and MFC₂ were also compared and the results are shown in **Figure 3c**. The comparison of maximum power densities in MFC₁ and MFC₂ indicated that the MFC₂ possesses approximately 17-70 % higher maximum power density. Increase in power density of MFC₂ can be related to decrease of internal resistance which is due to variation of properties in membrane and electrode surfaces. The membrane with higher surface area in MFC₁ induced undesirable effects on power density (36, 37).

4. Discussion

The types of substrate, microorganism and the reactor that led to increase in power density of MFC, are identified. It should be determined which variable have greater impact on the power density of MFC. To clarify the effect of each of these different variable factors on the power density, a simple three symbol notation was used. The first symbol indicates the type of substrate, second symbol shows the type of microorganism and the third symbol is related to type of reactor (order of notation is as follows: “S, type of substrate” “M, type of microorganism” “R, type of reactor”). There are two subscripts for each variable and they are determined as “a” and “b” when used hereafter subscript “a” refers to lower power density and subscript “b” refers to higher power density of MFCs (**Fig. 4**). For substrate, “a” represents glucose and “b” represents acetate. In the case of microorganism, “a” indicates *S. cerevisiae* and “b” shows *Shewanella* and similarly in the case of reactor design, “a” represents the MFC₁ and “b” represents the MFC₂. As an example, S_aM_bR_a shows that MFC₁ has been used with *Shewanella* culture and the glucose as substrate.

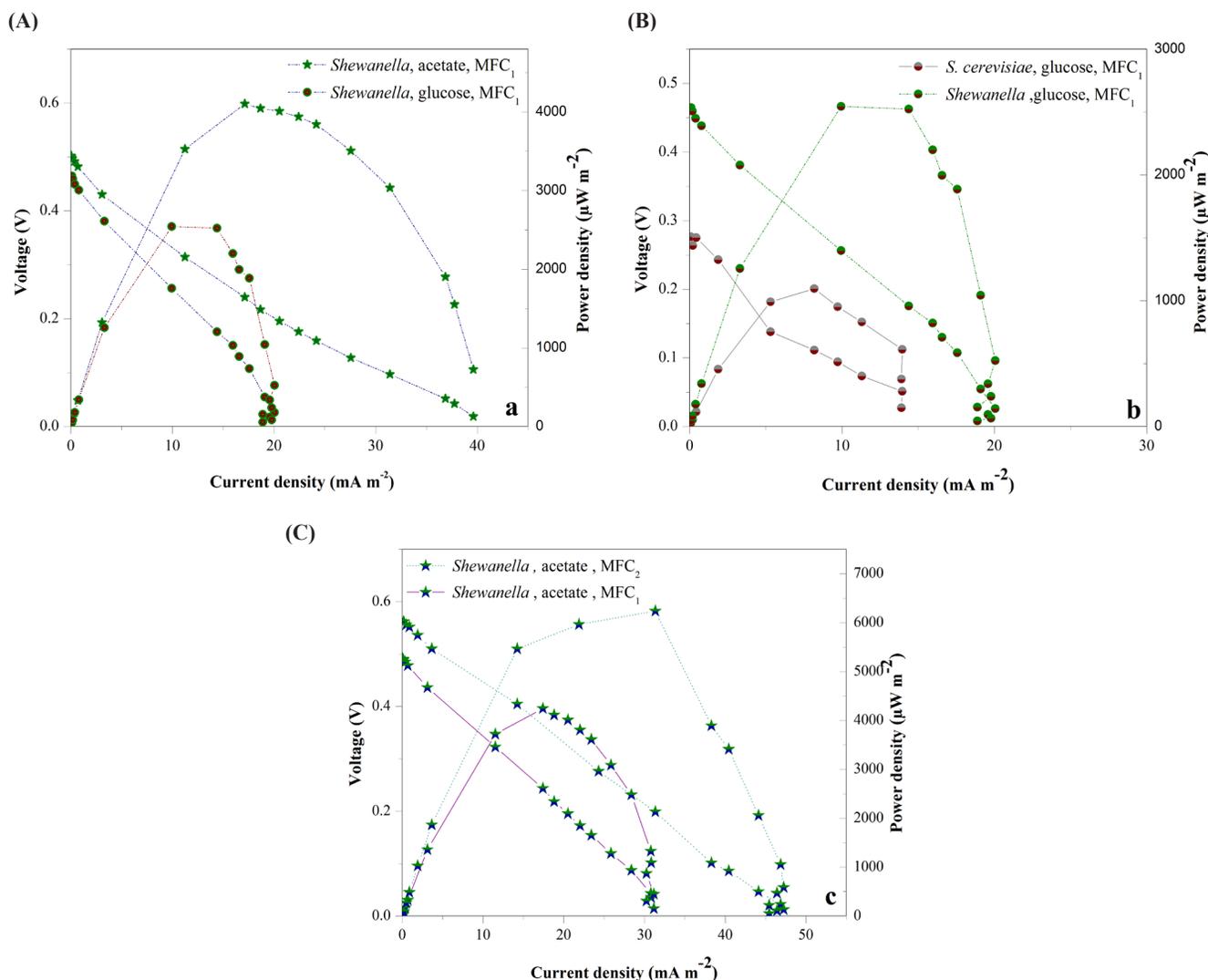


Figure 3. (A) Polarization curves and I-V curves with respect to the different substrate types in presence of *S. cerevisiae*. (B) Polarization curves and I-V curves at different microbial communities (C) MFC performance (power density) with respect to the different reactor design

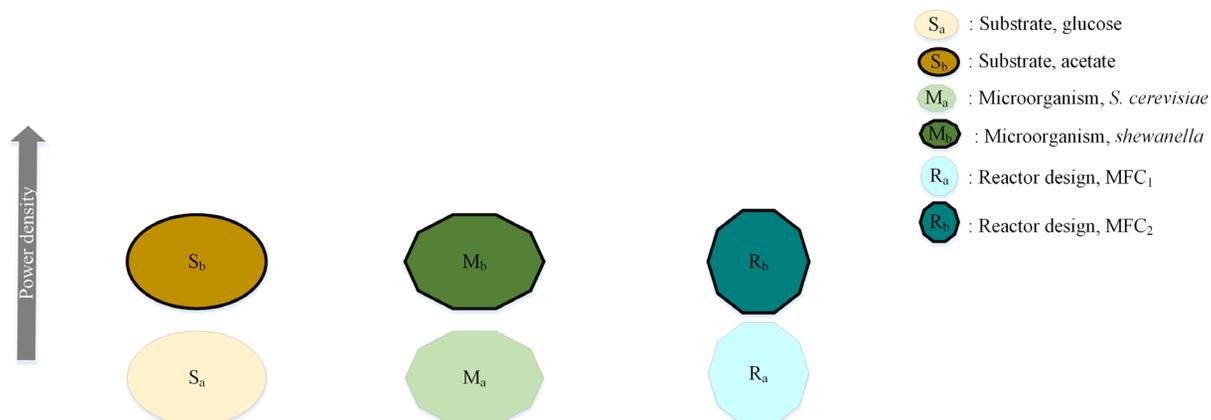


Figure 4. The schematic figure for illustration the used symbols

By considering all the possibilities, eight cases have been examined in this investigation and their power density values for each combination is obtained (Table 2). Among eight combination of substrate,

microorganism and reactor design type $S_bM_bR_b$ combination possessed the highest power density. To discern which of these three variables (i.e. substrate, microorganism and reactor design) had the most

effective role in maximizing the power density, $S_bM_bR_b$ (maximum power density production) is compared with the cases in which one of the variables has led to lower power density. Thereby, the power densities of MFCs in the $S_bM_bR_a$, $S_bM_aR_b$ and $S_aM_bR_b$ have been compared with that of $S_bM_bR_b$. The differences in the power densities of $S_bM_bR_a$, $S_bM_aR_b$ and $S_aM_bR_b$ with $S_bM_bR_b$ are $2100 \mu W m^{-2}$, $1500 \mu W m^{-2}$ and $200 \mu W m^{-2}$, respectively (Fig. 5a).

According to the results, the difference between the power density of $S_bM_bR_b$ and $S_bM_bR_a$ is more than those of other cases. In the other words, the MFC with second type of reactor i.e. “b” resulted higher power density and also the effect of reactor type on the power density was more than those of other variables. Or it can be mentioned that varying the reactor components for optimizing the MFC can induce prominent effect on power density. Concentration polarization, ohmic

losses, a thick non-conductive biofilm, hydrodynamics and geometrical aspects of the cell design would be a noticeable factor in performance of MFCs (5, 38).

For more clarity, the case of $S_aM_aR_a$ in which all variables yield lower power density can be considered and compared. The differences in the power density of $S_aM_aR_b$, $S_aM_bR_a$ and $S_bM_aR_a$ with $S_aM_aR_a$ are $2685.4 \mu W m^{-2}$, $1449 \mu W m^{-2}$ and $1005.3 \mu W m^{-2}$, respectively (Fig. 5b). In this case also the difference of the power density of $S_aM_aR_a$ and $S_aM_aR_b$ is more than others. It can be concluded that the improvement of the MFC reactor design, even without optimizing two other factors, could remarkably increase the power density. Modification of reactor design even when other parameters are not optimized can increase power density more than three times. However, the role of microorganisms to transfer electrons to the anodes and the effect of substrate on generation of electrons cannot be forgotten.

Table 2. Comparison of substrates in the MFCs enriched with the different microbial communities

Substrate	Microorganism	Type of MFC	Notation symbol	Max Voltage (mV)	Max power density ($\mu W m^{-2}$)
1 Acetate	<i>S. cerevisiae</i>	MFC ₁	($S_b M_a R_a$)	456	2100.8
2 Glucose	<i>S. cerevisiae</i>	MFC ₁	($S_a M_a R_a$)	405	1095.5
3 Acetate	<i>Shewanella</i>	MFC ₁	($S_b M_b R_a$)	507	4104.1
4 Glucose	<i>Shewanella</i>	MFC ₁	($S_a M_b R_a$)	499	2544.5
5 Acetate	<i>S. cerevisiae</i>	MFC ₂	($S_b M_a R_b$)	549	3153.2
6 Glucose	<i>S. cerevisiae</i>	MFC ₂	($S_a M_a R_b$)	520	3753.9
7 Glucose	<i>Shewanella</i>	MFC ₂	($S_a M_b R_b$)	513	5973.1
8 Acetate	<i>Shewanella</i>	MFC ₂	($S_b M_b R_b$)	592	6237.5

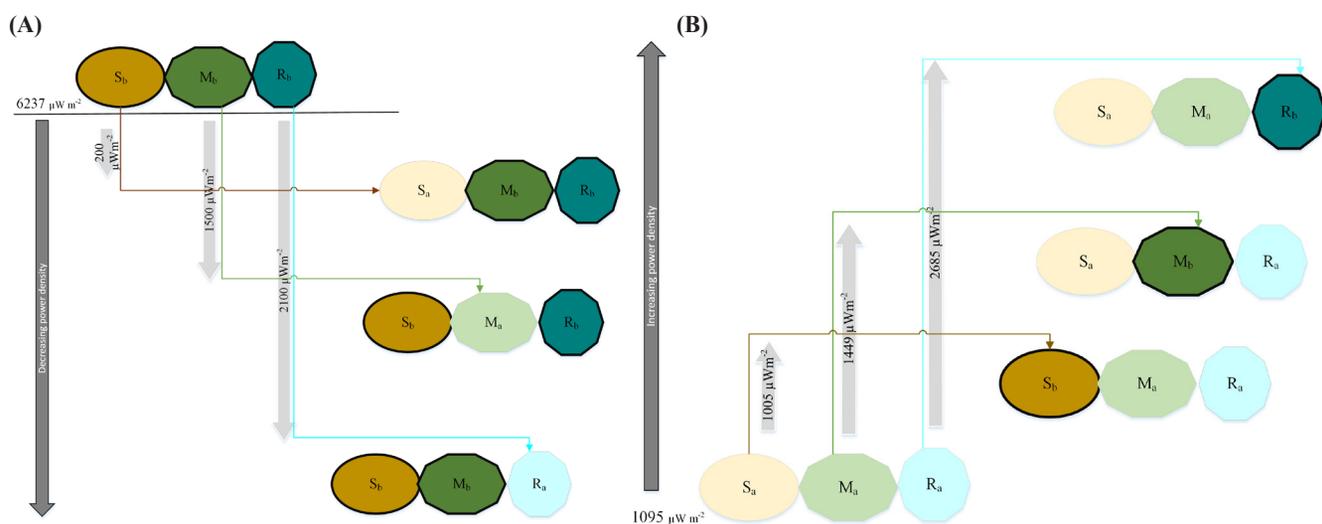


Figure 5. (A) The schematic figure for a case which all the variables are optimized and comparing it with cases that one variable is not optimized. (B) The schematic figure for a case which none of the variables are optimized and comparing it with cases that one variable is optimized

6. Conclusion

In this work different physical-chemical properties such as operating and design conditions affecting the

power generation were studied in order to optimize the overall performance of MFC. In general, considering the results, it seems that among the different factors,

reactor components have had significant and the most important effect on performance of the MFC while other factors were also determining.

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