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Evaluation of Bacterial Strains for Bio-Hydrogen Production from Domestic Wastewater in Microbial Electrolysis Cells

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ABSTRACT

Microbial electrolysis cells (MECs) are important for environment and renewable energy source. They were used for bio-hydrogen production by bacteria from organic matters, which is biological method to treat wastewater. Therefore, in this study MEC was used for bio-hydrogen producing by two bacterial strains: *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 in MEC 1 (300ml), MEC 2 (400ml) and MEC 3 (500ml) were applied from domestic wastewater. Volumes of MEC refer to anode chamber. Applied voltage of 0.4V, 0.6V and 0.8V was used as an external electrical circuit in MECs (1, 2 & 3). The lowest value of Bio-Hydrogen production rate (Bio-HPR) 112.28 cm³ was obtained by domestic wastewater without bacteria in MEC 3 (500ml) at applied voltage 0.8V. While highest values of Bio-HPR 235.87 and 268.08 cm³ were obtained by *E. coli* NRRL B-3008 and *P. aeruginosa* ATCC 27853 in MEC 3 (500ml) at applied voltage 0.8V from domestic wastewater respectively.

Keywords: Bio-hydrogen, Bacterial strains, Domestic wastewater, Microbial Electrolysis Cells.

INTRODUCTION

Hydrogen (future fuel) is the most important renewable energy sources in the 21st century. It has many properties: broad specific energy, large storage capacity, high energy conversion, and zero emissions (Environment friendly). Hydrogen was used in industrial processes: Oil refinery and ammonia industrial. Hydrogen production is receiving great global attention (AlZahrani and Dincer 2021).

Many bacteria have been used to produce bio-hydrogen in MEC: *Geobacter* sp., *Shewanella* sp., *Pseudomonas* sp., *Desulfuromonadales* spp., *Clostridium* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes* and *Lactobacillus* sp. Bacteria decomposed and oxidized organic matter present in domestic wastewater. Bacteria that catalyze the anode reactions, release of electrons, and biofilm formation in MECs are called electroactive bacteria (Patwardhan *et al.*, 2021). Hydrogen was produced by fossil fuel sources accounts for 96% of global production. The disadvantages of this method of producing hydrogen include high energy consumption and causing environmental pollution. Biological methods are alternative methods for hydrogen gas production from renewable environmental sources using biomass (Parkinson *et al.*, 2019).

Many methods were used for hydrogen fuel production from biomass, wastewater and waste materials by algae, bacteria and cyanobacteria at the ambient temperature and pressure. Biological methods were used for bio-hydrogen production includes photolysis, photo/dark fermentation and MEC. Hydrogen was produced by biological methods is called bio-hydrogen (Kadier *et al.*, 2016).

MEC is belonging to Bioelectrochemical systems (BES) used to produce bio-hydrogen by biological method. Bio-hydrogen was produced in MEC from many substrates:

glucose, glycerol, acetate and various biomass sources such as agricultural waste, wood waste, forest waste, lignin, cellulose, domestic/industrial wastewater. Bacterial strains and applied voltage were used in MEC to oxidation of organic matter for electrons, protons and carbon dioxide production (Sharma *et al.*, 2022).

MEC, whether it is a single or double chamber, does not differ in the principle of work. Double chamber MEC consists from anode chamber which contents anode electrode (positive electrode) and cathode chamber which contents cathode electrode (negative electrode). Proton exchange membranes (PEM) or Salt Bridge that is separate between anode and cathode chamber. Also PEM (or salt bridge) plays important role in MEC because prevents hydrogen diffusion to anode chamber again (Muddasar *et al.*, 2022).

MEC operation relies on the presence of bacteria and power supply as external applied voltage. At the reaction chamber (anode chamber) electrons, protons and carbon dioxide produce from organic matter in wastewater were decomposed and oxidized by bacteria. Bacteria were released electrons anode chamber. Electrons were collected at anode electrode and transferred to cathode chamber by external electrical circuits. Protons were transferred to cathode chamber by membrane (salt bridge) to combining with electrons for bio-hydrogen production (Park *et al.*, 2022). Applied voltage was used for bio-hydrogen in MEC about 0.2–0.9 V. Bio-hydrogen was produced under anaerobic conditions in MEC. Anode, cathode and proton exchange membranes (PEM) are very important ingredients because they are affecting in performance and operation of bioelectrochemical systems (BES); microbial fuel cell (MFC) and MEC (Abd-Elrahman *et al.*, 2022). The physical and chemical properties of hydrogen gas are presented in Table 1.

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Table 1. The Physical and chemical properties of hydrogen gas (Shchepakina et al., 2023).

Physical properties		Chemical properties	
	Hydrogen atom	Chemical Symbol	H
Atomic number	1	Formula	H ₂
Atomic weight	1.00782519 (on 12C scale)	Chemical structure	H-H
CAS Registry Number:	12385-13-6	Molecular weight	2.0159
	Hydrogen molecule	CAS Registry Number	1333-74-0
Electron configuration	1s	UN Number	Gas = UN1049 Liquid = UN1966
Density (gas)	0.090 g/L	RTECS Number	MW 8900 000
Density (liquid)	0.70g/L	Appearance	Odorless gas at room temperature and colorless
Density at 273 K	(kg/m ³) 0.09	Molar mass	2.016 (kg/kmol)
Relative vapor density (air = 1)	0.07	Energy content (1 kg H ₂)	H ₂ + O ₂ → 2H ₂ O
Melting point	-259.35 °C	Heating values	Higher = 141 900 kJ; 33 900 kcal; 39.4 kWh Lower = 120 000 kJ; 28 680 kcal; 32.9 kWh
Boiling point	-252.88 °C (at 1 atm)		
Ionic radius (H)	1.54 Å		
van der Waal radius	1.2 Å		
Ionization potential	13.598 eV		
Pauling electronegativity	2.1		
Solubility in water	0.0214 cm ³ / g (0 °C, 1 atm)		
Diffusivity in air	0.63 cm ² / g		
Flammability range	4 – 75 % (in air)		
Auto-ignition temperature	500 – 571 °C		

In this work, three types of MECs (MEC 1 (300ml), MEC 2 (400ml) and MEC 3 (500ml)) were used to produce bio-hydrogen. Domestic wastewater was used as a substrate for bio-hydrogen in MECs. Studying the effect of two species of bacterial strains (*E. coli* NRRL B-3008 and *P. aeruginosa* ATCC 27853) for stimulate anode reactions and release of electrons. Applied voltage 0.4V, 0.6V and 0.8V were used for electron transfer to cathode electrode.

MATERIALS AND METHODS

Source of bacterial strains

Bacterial strains (*E. coli* NRRL B-3008 and *P. aeruginosa* ATCC 27853) were used for Bio-hydrogen production and obtained from MIRCEN (Microbial Resource Cen., Fac. of Agric.) Ain Shams Univ., Egypt. Bacterial strains were prepared by nutrient broth medium (OXOID Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, CM0001, UK) according to Abd-Elrahman, (2017)

Domestic wastewater

Domestic wastewater were used as substrate obtained from EL-Khanka (EL-Beraka wastewater treatment plant), EL-Qaliubiya, Egypt. To adjust the pH of domestic wastewater to 7 was used drops of 1M HCl and buffer solution (sodium phosphate 0.2M). Domestic wastewater contents of organic matter 16.35 % and COD 243 mg /L pH= 7.4 (Afify, et al. 2017).

MEC setup/operation

MEC was used as bioreactor consists of two-chamber (Anode and Cathode chambers) H-cell type reactors. Three MECs were Designed, MEC 1 (with a working volume of 300 mL per chamber), MEC 2 (volume of 400 mL) and MEC 3 (volume of 500 mL), separated by salt bridge (KCl (1M) + Agar 20%) as membrane and were used for all experiments. Carbon brush was used as anode. Stainless steel (304) was used as cathode. Anode and cathode electrode (or chambers) were connected by copper wire support by applied voltage (0.4, 0.6 & 0.8 V). In MEC 1, MEC 2 and MEC3 Anode chambers were filled with substrate (domestic wastewater) at 300 ml, 400 ml and 500 ml depending on the chamber volume. Cathode chamber was filled by distilled water in MEC 1, MEC 2 and MEC3, 300ml, 400ml and 500ml depending on the chamber

volume. Bacterial cultures were added to the surface of carbon (anode electrode) to stimulate the work of electrode, transfer electrons, and form the biofilm.

Measurement of Bio-hydrogen production rate (Bio-HPR cm³)

Bio-hydrogen gas was produced in cathode chamber. Downward displacement of water method was used for collected bio-hydrogen gas production (Ujwal et al., 2015).

$$\text{Bio-HPR} = \text{Burette reading length (cm)} \times \pi r^2 (\text{cm}^2) = (\text{V cm}^3)$$

Statistical analysis

Statistix 9 was used for statistical analysis of data at LSD 5%.

RESULTS AND DISCUSSION

Control experiment

Domestic wastewater contains millions of aerobic, facultative and anaerobic bacteria. Facultative bacteria oxidize and decompose organic materials present in domestic wastewater into simpler intermediates and also use free oxygen as an electron acceptor. The applied voltage was used in the experiment transfer electrons from anode to cathode chamber (Koul et al., 2022).

In this experiment, bio-hydrogen was produced using domestic wastewater based on the bacteria present in it. MEC 1 (300 ml), MEC 2 (400 ml) and MEC3 (500 ml) were filled with domestic wastewater in anode chamber and distilled water were filled in cathode chamber. In MEC 1 (300 ml) production of the Bio-hydrogen gas in cathode chamber were started in 6th day at applied voltage 0.6 and 0.8 V while were started in 7th day with applied voltage 0.4 V at applied voltage 0.8 V was obtained the highest value for Bio-hydrogen production rate (Bio-HPR) 54.64 cm³ while at applied voltage 0.4 V was obtained the lowest value for Bio-HPR 41.49 cm³ which indicates that there is a significant difference between them. Values of Bio-HPR 54.64 cm³ and Bio-HPR 52.11 cm³ at applied voltage 0.6 V, It indicated that there are no significant differences (Fig. 1).

In MEC 2 (400 ml) the Bio-hydrogen gas was started production in 4th day at applied voltage 0.6 V and 0.8 V. While in the case of using applied voltage 0.4 V the Bio-

hydrogen gas was started production in 5th day. Highest value for Bio-HPR 90.57 cm³ at applied voltage 0.8 V and value for Bio-HPR 87.03 cm³ were obtained at applied voltage 0.6 and 0.8 V they indicated that there are no significant differences. The lowest value for Bio-HPR 74.88 cm³ at applied voltage 0.4 V was significantly greater than other values of Bio-HPRs (Fig. 2).

In MEC3 (500 ml) Bio-hydrogen gas was started production in 4th day at all applied voltage, highest value of Bio-HPR 112.83 cm³ at applied voltage 0.8 V, which indicates that there is a significant difference was found between this value and other values. No significant difference was found between value of Bio-HPR 98.16 cm³ at applied voltage 0.6 V and Bio-HPR 95.12 cm³ is the lowest value (Fig. 3).

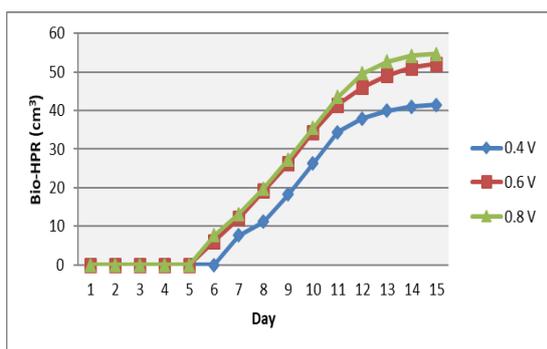


Fig.1. Bio-HPR (cm³) from domestic wastewater in MEC 1 (300 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 3.57).

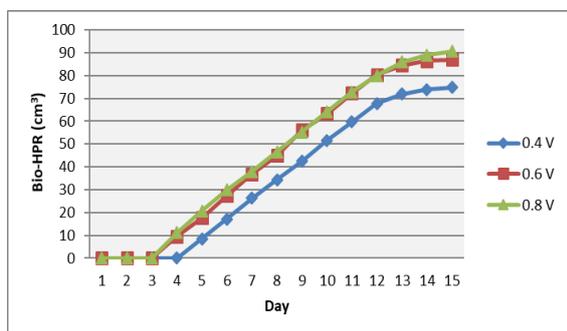


Fig.2. Bio-HPR (cm³) from domestic wastewater in MEC 2 (400 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 5.84).

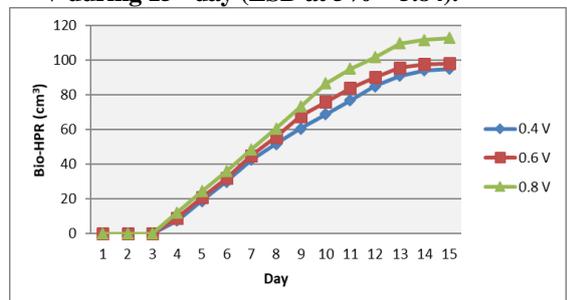


Fig.3. Bio-HPR (cm³) from domestic wastewater in MEC 3 (500 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 4.42).

From the above, it is clear that the rates of bio-hydrogen was produced from domestic wastewater using MEC 1 (300 ml), MEC 2 (400 ml) and MEC3 (500 ml) differ depending on the volume of the anode chamber. The applied voltage 0.4, 0.6 and 0.8 V was used also led to

different of Bio-hydrogen production rates. Khan *et al.*, (2017) were consistent with these results, who investigated the possibility of producing bio-hydrogen gas from wastewater in MECs, also wastewater treatments during bio-hydrogen production.

***Escherichia coli* NRRL B-3008**

E. coli NRRL B-3008 is one of the electroactive bacteria that was used in MEC for bio-hydrogen production from wastewater. *E. coli* NRRL B-3008 bacteria have the ability to decompose and oxidize organic matter present in wastewater. Organic matter mainly consists of proteins, fats and carbohydrates. *E. coli* NRRL B-3008 uses carbohydrates as carbon source. Bacterial growth on the electrode leads to the formation of biofilms. Also, bacteria stimulate the anode reactions, which lead to the surface release of electrons. Applied voltage has the ability to transfers electrons to cathode (Liu *et al.*, 2012).

Bio-HPR (cm³) by *E. coli* NRRL B-3008 from domestic wastewater

Bacterial cultures of *E. coli* NRRL B-3008 were added to the surface on anode electrode at rates of 30 ml to MEC 1 (300 ml), 40 ml to MEC 2 (400 ml) and 50 ml to MEC 3 (500 ml). The results were obtained when operating MEC 1 (300 ml) after adding bacterial cultures in the previous ratios showed that, the Bio-hydrogen gas was started production in 4th day at applied voltage 0.6 and 0.8 V and in 5th day at applied voltage 0.4 V. onwards until to 15th day. At applied voltage 0.8 V was obtained the highest values for Bio-HPR 145.22 cm³ and was obtained the lowest value for Bio-HPR 110.3 cm³ at applied voltage 0.4 V, Which indicates that there is a significant difference between these values (Fig.4).

On the other hand, The Bio-hydrogen gas was started production in 2nd day at applied voltage 0.6 V at MEC 2 (400 ml) and 3rd day with applied voltage 0.4 and 0.8 V. Highest value for Bio-HPR 207.96 cm³ at applied voltage 0.8 V was significantly lower than value of Bio-HPR 204.42 cm³ at applied voltage 0.6 V and lowest value for Bio-HPR 200.37 cm³ at applied voltage 0.4 V (Fig.5). In the recent experiment using bacterial cultures of *E. coli* NRRL B-3008 in MEC 3 (500 ml), it was found that the rates of Bio-hydrogen production were as follows: Production of Bio-hydrogen gas was started in 2nd day at applied voltage 0.6 and 0.8 V while in 3rd day at applied voltage 0.4 V, highest value of Bio-HPR 235.87 cm³ at applied voltage 0.8 V. at applied voltage 0.4 V was obtained the lowest value for Bio-HPR 221.12 cm³, which indicates that there is a significant difference was found between them (Fig.6). Bio-HPR (cm³) was produced by *E. coli* NRRL B-3008 from domestic wastewater in MEC 1 (300 ml), MEC 2 (400 ml) and MEC 3 (500 ml) which increasing in 5th and 6th day because the biofilm was formed around on the surface of the anode electrode which indicates *E. coli* NRRL B-3008 growth, which increasing the rate of organic matter degradation. Figs. (4, 5 & 6) present the Bio-HPR (cm³) which was produced by *E. coli* NRRL B-3008 from domestic wastewater in MEC 1 (300 ml), MEC 2 (400 ml) and MEC 3 (500 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day.

These results are in the line with those obtained by Samsudeen *et al.* (2020) who reported that *E. coli* was able to degrade and oxidize organic matter in wastewater and release electron when biofilm formation on anode electrode.

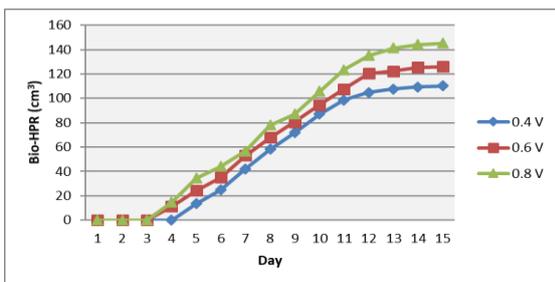


Fig.4. Bio-HPR (cm³) by *E. coli* NRRL B-3008 from domestic wastewater in MEC 1 (300 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 4.36).

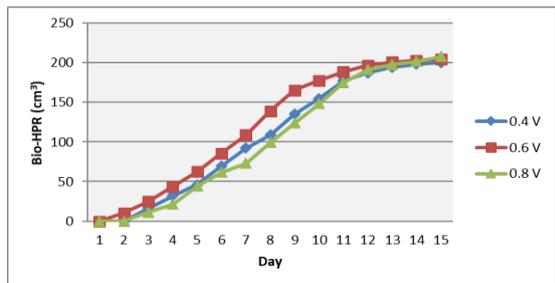


Fig.5. Bio-HPR (cm³) by *E. coli* NRRL B-3008 from domestic wastewater in MEC 2 (400 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 3.79).

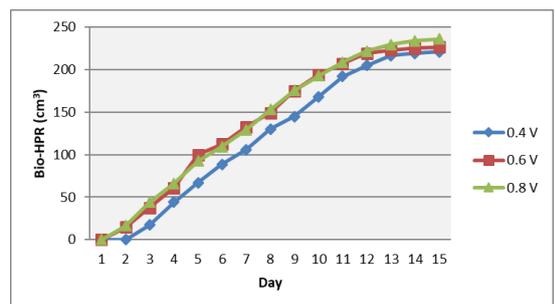


Fig.6. Bio-HPR (cm³) by *E. coli* NRRL B-3008 from domestic wastewater in MEC 3 (500 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 4.91).

***Pseudomonas aeruginosa* ATCC 27853**

Also, *P. aeruginosa* is electroactive bacteria that have the ability to decompose and metabolize organic matter present in wastewater as carbon and nitrogen sources. For bio-hydrogen gas production by *P. aeruginosa* ATCC 27853, domestic wastewater was used as a source of organic matter in MEC 1 (300 ml), MEC 2 (400 ml) and MEC 3 (500 ml) at applied voltage 0.4, 0.6 and 0.8 V.

Bio-HPR (cm³) by *P. aeruginosa* ATCC 27853 from domestic wastewater

Bacterial cultures of *P. aeruginosa* ATCC 27853 were added in the same rates as bacterial cultures of *E. coli* NRRL B-3008, 30 ml to MEC 1 (300 ml), 40 ml to MEC 2 (400 ml) and 50 ml to MEC 3 (500 ml). Bacterial cultures of *P. aeruginosa* ATCC 27853 must be added to the surface of the anode in order to stimulate oxidation and reduction reactions (anodic reactions) in anode chamber and release electrons. Also, adding bacterial cultures to the anode surface stimulates the formation of biofilm. In MEC 1 (300 ml) of the results obtained it was found that, at applied voltage 0.4 V was obtained the highest value of Bio-HPR 159.89 cm³ which indicates that there is a

significant difference with the obtained values for Bio-HPR at applied voltage 0.6 and 0.8 V, which started in 3rd day. There are no significant differences between the values of Bio-HPR 151.8 cm³ and Bio-HPR 146.23 cm³ at applied voltage 0.6 and 0.8 V consecutively, which started in 4th day (Fig. 7).

Fig. (8) presents the Bio-HPR in MEC 2 (400 ml) with a variable applied voltage. In 2nd day the bio-hydrogen gas was started production at applied voltage 0.4 V and in 3rd day at applied voltage 0.6 and 0.8 V. Highest value for Bio-HPR 193.79 cm³ at applied voltage 0.8 V. The results indicate that there is significant differences were found between this highest value for Bio-HPR and other values. The Bio-HPR 179.63 cm³ is the lowest value was obtained at applied voltage 0.4 V.

In MEC 3 (500 ml), the obtained results indicated that, also in 2nd day the bio-hydrogen gas was started production at all applied voltage on this experiment. Highest value of Bio-HPR 268.08 cm³ at applied voltage 0.8 V. At applied voltage 0.4 V was obtained the lowest value for Bio-HPR 232.76 cm³. The highest value for Bio-HPR was obtained indicates that there are significant differences between it and the values were obtained at applied voltage 0.4 and 0.6 V in MEC 3 (500 ml) (Fig. 9).

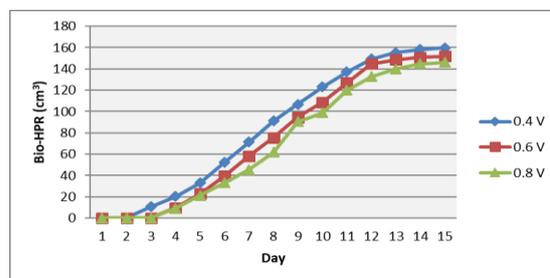


Fig.7. Bio-HPR (cm³) by *P. aeruginosa* ATCC 27853 from domestic wastewater in MEC 1 (300 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 6.83).

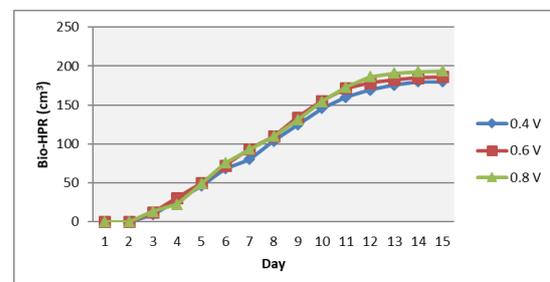


Fig.8. Bio-HPR (cm³) by *P. aeruginosa* ATCC 27853 from domestic wastewater in MEC 2 (400 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 5.78).

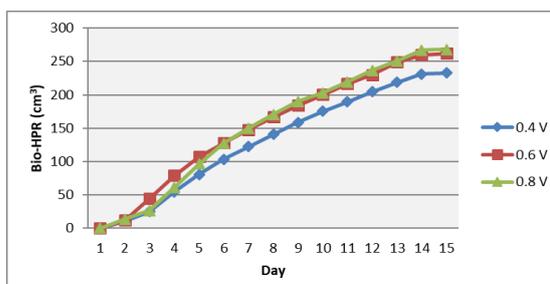


Fig.9. Bio-HPR (cm³) by *P.s aeruginosa* ATCC 27853 from domestic wastewater in MEC 3 (500 ml) at applied voltage 0.4, 0.6 and 0.8 V during 15th day (LSD at 5% = 5.86).

These results are confirmed with Hua *et al.*, (2019) where they also used bacterial strain such as *Pseudomonas aeruginosa* to produce bio-hydrogen gas in MEC and wastewater treatment.

CONCLUSION

To evaluate the role of bacterial strains in producing bio-hydrogen gas from domestic wastewater using MECs, it was found that: facultative anaerobic bacteria present in wastewater have an effective role in using free oxygen as an electron acceptor during anode reactions. When *E. coli* NRRL B-3008 were added to wastewater, a significant improvement was found in Bio-HPR compared to the use of wastewater without the addition of bacteria. *P. aeruginosa* ATCC 27853 achieved higher Bio-HPR than *E. coli* NRRL B-3008. Bio-HPRs were affected by several factors, including bacterial strains, applied voltage and volume of MEC.

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تقييم السلالات البكتيرية لإنتاج الهيدروجين الحيوي من مياه الصرف الصحي المنزلية في خلايا التحليل الكهربائي الميكروبية

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^٢قسم خصوبة وميكروبيولوجيا الأراضى - مركز بحوث الصحراء - المطرية - القاهرة - مصر

الملخص

تعتبر خلايا التحليل الكهربائي الميكروبية (MECs) مهمة للبيئة ومصدرًا للطاقة المتجددة. يتم استخدامها لإنتاج الهيدروجين الحيوي بواسطة البكتيريا من المخلفات العضوية، وهي تقنية فعالة في معالجة مياه الصرف الصحي المنزلية. ولذلك، في هذه الدراسة تم استخدام خلايا التحليل الكهربائي الميكروبية (MECs) لإنتاج الهيدروجين الحيوي بواسطة سلالتين من البكتيريا: (*Escherichia coli* NRRL B-3008 و *Pseudomonas aeruginosa* ATCC 27853). في MEC 1 (٣٠٠ مليلتر)، MEC 2 (٤٠٠ مليلتر) و MEC 3 (٥٠٠ مليلتر). تم استخدام جهد كهربى خارجى ٠,٤ فولت، ٠,٦ فولت و ٠,٨ فولت. تم الحصول على أقل قيمة لمعدل إنتاج الهيدروجين الحيوي (Bio-HPR) وهو ١١٢,٢٨ سم^٣ من مياه الصرف الصحي المنزلية دون إضافة البكتيريا في MEC 3 عند جهد كهربى خارجى ٠,٨ فولت. في حين تم الحصول على أعلى قيمة لمعدل إنتاج الهيدروجين الحيوي (Bio-HPR) وهو 235.87 سم^٣ و ٢٦٨,٠٨ سم^٣ بواسطة بكتيريا *E. coli* NRRL B-3008 و *P. aeruginosa* ATCC 27853 في MEC 3 عند جهد كهربى خارجى ٠,٨ فولت من مياه الصرف الصحي المنزلية على التوالي.