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Chemical Oxygen Demand (COD) Removal and Hydrogen Yield in Microbial Electrolysis Cells

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BIO-HYDROGEN production and wastewater treatment are two processes that take place simultaneously within microbial electrolysis cells (MEC). Therefore, in this study, MEC1 (300ml), MEC2 (400ml) and MEC 3 (500ml) were used as three different volumes to produce bio-hydrogen, and the ability of each of them to treat wastewater was evaluated. Also, three different types of bacterial strains (*Escherichia coli* NRRL B-3008, *Enterobacter aerogenes* DSM 30053 and *Pseudomonas aeruginosa* ATCC 27853) were used to catalyze the anode reactions in the MEC. The applied voltage was used to operate MEC is 0.4, 0.6 and 0.8 V. The wastewater was intended for treatment is domestic wastewater and industrial wastewater. The results were based on estimating the values of Bio-hydrogen production rates (Bio-HPR), hydrogen yield (YH_2 %), and Chemical oxygen demand (COD) removal efficiency (%). In MEC 3 (500 ml) at applied voltage 0.8 V and industrial wastewater was gave the highest rates of values for Bio-HPR 358.24cm^3, YH_2 93.64% and COD removal efficiency 88.04% were obtained by *Escherichia coli* NRRL B-3008. The lowest values were obtained from MEC1 (300 ml) at applied voltage 0.4 V were Bio-HPR 41.49 cm^3, YH_2 16.15 % and COD removal efficiency 67.07% by domestic wastewater without bacteria.

Keywords: Microbial Electrolysis Cell (MEC), Bacterial strains, Bio-hydrogen, COD removal efficiency (%), Applied voltage, Domestic wastewater, Industrial wastewater.

1. Introduction

Bio-electrochemical systems (BESs) such as microbial electrolysis cells (MECs) have the potential to produce bio-hydrogen in renewable and sustainable methods using organic matters and wastewater. There are many biological applications for MECs such as bio-hydrogen and bio-methane production, wastewater treatment and biosensors (Muddasar et al. 2022; Koriem et al. 2022**).**

The mechanism of microbial electrolysis cells for the production of biofuels and wastewater treatment is summarized in the oxidation and reduction reactions of the organic matter present in the wastewater. The chemical energy generated from the organic matter present in wastewater is 9.3 times greater than the energy required to treat it. MEC operates under anaerobic conditions to produce biohydrogen in a similar method to anaerobic digestion methods for methane production and wastewater treatment (Fudge et al. 2021).

The design of the MEC includes the presence of electrodes (anode and cathode) balanced with specific measurements, and bacteria are used as biocatalysts to activate the oxidation and reduction reactions of the biomass inside the anode chamber. Bacteria have a very important role in forming the biofilm on the anode surface. The biofilm has the ability to convert the chemical energy contained in organic materials into bio-hydrogen or bio-methane. The bacteria used to operate the MEC are called electrochemically active bacteria, and therefore have the ability to transfer electrons between electrodes (Dincer and Acar 2015).

MECs designed for bio-hydrogen production and wastewater treatment require a separator between the anode and cathode called an ion exchange membrane (IEM). The anode and cathode are connected to an external power source called an applied voltage. Domestic wastewater and industrial wastewater used as a substrate are added to operate the MEC under anaerobic conditions. Bacteria are added to the surface of anode and an external power source is connected to initiate oxidation and reduction reactions of the organic matter inside the anode

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chamber. This produces bio-hydrogen and wastewater treatment at the same time (Bajracharya et al. 2016).

There are many factors that affect the efficiency of MEC performance in bio-hydrogen production and wastewater treatment, such as the type of MEC, substrate, bacterial strain, applied voltage, type of electrode, pH and temperature. Type of MEC include single or double chamber. Substrate such as: sodium acetate, glycerol, domestic wastewater and industrial wastewater. Bacterial strains called electrochemically active bacteria, such as: *Shewanella* sp., *Geobacter* sp., *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Clostridium* sp. *Desulfuromondales* sp., *Dysgomonas* sp. and *Bacteroides* sp. Applied voltage ranged between 0.2V to 0.9V, pH about 6 – 7.5. The temperature ranges between 30 –37 °C (Abd-Elrahman et al. 2022a and Elsakhawy et al. 2017**).**

The present study therefore aims to use three different volumes of MEC (MEC1 300 ml, MEC2 400 ml and MEC3 500 ml) at applied voltages 0.4, 0.6 and 0.8 V. Using wastewater (domestic and industrial wastewater) as a substrate in double chambers of MEC chambers. Bacterial strains (*Escherichia coli* NRRL B-3008, *Enterobacter aerogenes* DSM 30053 and *Pseudomonas aeruginosa* ATCC 27853) were used for decomposing organic materials, forming biofilm on the surface of the anode and releasing electrons. The role of volumes MEC, substrates and bacterial strains in their compatibility in bio-hydrogen production and wastewater treatment has been scientifically verified through parameters that are Bio-hydrogen production rates (Bio-HPR), hydrogen yield (YH2 %) and Chemical oxygen demand (COD) removal efficiency (%).

2. Materials and methods

Source of bacterial strains

Escherichia coli NRRL B-3008, *Enterobacter aerogenes* DSM 30053 and *Pseudomonas aeruginosa* ATCC 27853 three bacterial strains were obtained from Faculty of Agriculture, MIRCEN, Ain Shams University, Cairo, Egypt. Nutrient broth medium (13 gm from medium/1 L distilled water) was used for preparation bacterial strains according to Afify et al. (2023a).

Substrates

Two types of substrates were used for Bio-hydrogen production in MEC. (1) Domestic wastewater was obtained from Wastewater treatment plant (EL-Beraka), EL-Khanka, EL-Qaliubiya, Egypt. (2) Industrial wastewater was obtained from small industrial area (Pickles Factory), EL-Obour, EL-Qaliubiya, Egypt. Chemical analyses of chemical oxygen demands (COD), organic matter and pH in substrates was carried out in Desert Research Centre (Central Lab). From the results obtained, it was found that the domestic wastewater contents of COD 243 mg /L, organic matter 16.35 % and pH= 7.4, while the content of industrial wastewater in COD is 276 mg /L, organic matter is 19.78 % and pH=4.4. Drops of HCl (1M) were added to domestic wastewater and drops of Na OH (1M) were added to industrial wastewater for adjusted pH to 7. Sodium phosphate buffer solution was used to keep pH = 7 (Abd-Elrahman et al. 2022b)

Microbial electrolysis cells (MECs)

MEC1, MEC2 and MEC3 are three different bioreactor designs for bio-hydrogen production. All MECs were used consists of two-chamber Anode and Cathode chambers. Volumes of anode/cathode chambers are MEC 1 (300 mL), MEC 2 (400 mL) and MEC 3 (500 mL). Salt bridge was used to separate between anode and cathode chambers. Salt bridge consists of Agar (20%) and salt (Potassium Chloride (1M)). The anode material was carbon brush (34 D) and the cathode electrode material was stainless steel (304).0.4, 0.6 and 0.8 V was used as applied voltage for all experiments. Substrates were filled in Anode chamber. Distilled water was filled in Cathode chamber. Anode and cathode electrode (or chambers) were connected by copper wire. Cultures of bacterial strains were added to the surface area of the anode electrode to stimulate the work of the electrode, transfer electrons and biofilm formation. (Abd-Elrahman et al. 2022b).

Calculations

Bio-hydrogen production rate (Bio-HPR cm^3)

In cathode chamber Bio-hydrogen gas was produced and collected according to the method of Afify et al. (2017b). The method that has been used to measure Bio-hydrogen production rates Bio-HPR (cm^3) is water displacement.

Bio-HPR (cm^3) = Reading of burette length (cm) × π r^2 (cm^2)

(π) = 3.14, (r) = burette tube's radius

Hydrogen yield (YH_2 %)

Amount of hydrogen was produced from a substrate called Hydrogen Yield (YH_2) and it is calculated through the following equation: **Logan *et al.* (2008)**.

$$YH_2 = \frac{{}^{n}H_2}{{}^{n}th} \times 100\ \%$$

${}^{n}H_2$ = the moles of hydrogen was produced. It is calculated through the following equation:

$${}^{n}\mathrm{H}_2 = \frac{\mathrm{Bio,HPR}}{\mathrm{R\,T}}$$

R = gas constant = 0.08314 L bar / K mol and (T) =303 K is the absolute temperature. ^{n}th = the moles were converted of substrates. ^{n}th was calculated by equation:

$${}^{n}\mathrm{th} = \frac{2(\mathrm{COD}_{in} - \mathrm{COD}_{out})}{MO_2}$$

Where: MO_2 =32 gm / mol (the molecular weight of oxygen). COD $_{in}$ is the concentration of COD in substrate at the beginning and COD $_{out}$ is the concentration of COD in substrate at the end. One mole of COD was removed from substrate turn into 2 mole of hydrogen

COD removal efficiency (%) is calculated by equation:

$$\text{COD removal efficiency (\%)} = \frac{(\mathrm{COD}_{in} - \mathrm{COD}_{out})}{\mathrm{COD}_{in}} \times 100\%$$

Statistical analyses

LSD at 5% test was collected by statistical analysis software Statistix (9)

3. Results and discussion

3.1. Control experiment

COD present in wastewater and industrial wastewater indicates the amount of oxygen required to decompose the organic matter in the water sample (mg L^{-1} or gm^{-3}). The value of the COD is directly proportional to the value of the oxidizable organic matter present in the wastewater. The COD value in wastewater increases with the increase of water-soluble pollutants (Ahammad et al. 2020 and Abbas et al. 2021**).**

Domestic wastewater and industrial wastewater contain a large percentage of sugars, proteins, fibers, nitrogen and phosphorus. These components increase the COD value, so the wastewater treatment process focuses on removing these components to reduce the COD percentage (Koul et al. 2022).

Two control experiments were conducted in this research. In the first experiment, the values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) were estimated using domestic wastewater as a substrate for operating the MECs without adding bacteria. Bio-hydrogen gas was produced in MEC1, MEC2 and MEC3 at cathode chamber with applied voltage 0.4, 0.6 and 0.8 V. Bio-HPR 112.83 cm^3, YH_2 40.47% and COD removal efficiency 72.83 % are the highest values were obtained in MEC 3 (500 ml) at applied voltage 0.8 V, while the lowest values were obtained from MEC1 (300 ml) at applied voltage 0.4 V were Bio-HPR 41.49 cm^3, YH_2 16.15 % and COD removal efficiency 67.07%, which indicates a significant difference. Table (1)

In the second experiment, the values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) were also estimated using industrial wastewater as a substrate for operating the MEC without adding bacteria. In MEC 3 (500 ml) at applied voltage 0.8 V were obtained of the highest values of Bio-HPR 165.46 cm^3, YH_2 71.94% and COD removal efficiency 52.89 % which revealed significant differences were found between this values and other were obtained from MEC 1(300 ml) and MEC 2 (400 ml) at various applied voltage. Bio-HPR 79.44 cm^3, YH_2 48.02 % and COD removal efficiency 38.04 % are the lowest values were obtained in MEC1 (300 ml) at applied voltage 0.4 V table (2)

The present results are in agreement with those reported by Marone et al. (2017) which used different types of industrial wastewater from the manufacture of fruit juice, cheese and sugar as a substrate in MEC for bio-hydrogen production. Who reached COD removal efficiency 79% at applied voltage of 0.2 V.

Table 1. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by domestic wastewater.

	MEC 1			MEC 2			MEC 3		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD $_{in}$ (mg/L)	243	243	243	243	243	243	243	243	243
COD $_{out}$ (mg/L)	80	78	78	74	71	71	69	69	66
COD removal efficiency (%)	67.07	67.9	67.9	69.54	70.78	70.78	71.6	71.6	72.83
${}^{n}H_2$ (mol)	1.64	2.06	2.16	2.97	3.45	3.59	3.77	3.89	4.47
^{n}th (mol)	10.18	10.31	10.31	10.56	10.75	10.75	10.87	10.87	11.06
Bio-HPR (cm^3)	41.49	52.11	54.64	74.88	87.03	90.57	95.12	98.16	112.83
YH_2 %	16.15	20.04	21.02	28.12	32.12	33.43	34.7	35.81	40.47

Bio-HPR (LSD at 5% = 7.92), YH_2 % (LSD at 5% = 2.7).

Table 2. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by industrial wastewater.

	MEC 1			MEC 2			MEC 3		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD $_{in}$ (mg/L)	276	276	276	276	276	276	276	276	276
COD $_{out}$ (mg/L)	171	165	163	157	149	146	142	139	130
COD removal efficiency (%)	38.04	40.21	40.94	43.11	46.01	47.1	48.55	49.63	52.89
$^nH_2$ (mol)	3.15	3.61	3.78	4.29	4.95	5.22	5.58	5.80	6.56
^{n}th (mol)	6.56	6.93	7.06	7.43	7.93	8.12	8.37	8.56	9.12
Bio-HPR (cm^3)	79.44	91.08	95.38	108.28	124.98	131.56	140.66	146.23	165.46
YH_2 %	48.02	52.09	53.58	54.12	62.47	64.24	66.63	67.76	71.94

Bio-HPR (LSD at 5% = 9.55), YH_2 % (LSD at 5% = 2.65).

3.2. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) by *Escherichia coli* NRRL B-3008

MEC uses the ability of electrochemically active microbes to grow on the surface of the anode to form a biofilm. Electrochemically active bacteria stimulate oxidation and reduction reactions in the anode chamber by oxidizing the organic matter to electrons, protons and carbon dioxide. Bacteria transfer electrons to the anode directly through nanowires attached to bacterial cell membrane proteins, or electrons are released from the bacteria to the anode by shuttle movement. Then the electrons are transferred from the anode to the cathode through external electrical circuit in the presence of a applied voltage 0.2 – 0.8 V. The protons transfer through the ion exchange membrane (salt bridge) to the cathode chamber. Protons combine with electrons for bio-hydrogen production (Varanasi et al. 2019)**.**

Table 3 presents the values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) were obtained in MECs from domestic wastewater by *Escherichia coli* NRRL B-3008. MEC 3 (500 ml) were gave the highest values of Bio-HPR 235.87 cm^3, YH_2 73.76 % and COD removal efficiency 83.53 % at applied voltage 0.8 V, which indicated no significant differences were found between this values and other values at the MEC2 (400 ml). Bio-HPR 110.3 cm^3, YH_2 39.11 % and COD removal efficiency 73.66 % are the lowest values were obtained in MEC1 (300 ml) at applied voltage 0.4 V, which indicates a significant difference were found between the lowest values and other values.

The values of both Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) were estimated when using industrial wastewater as a substrate for production Bio-hydrogen gas by *Escherichia coli* NRRL B-3008 in MECs. The obtained results indicated that, Bio-HPR 358.24cm^3, YH_2 93.64% and COD removal efficiency 88.04% are the highest values were obtained in MEC 3 (500 ml) at applied voltage 0.8 V which referring significant differences were found between this results and other results were obtained in MEC 1 (300 ml) and MEC 2 (400 ml) at all applied voltage. The results were obtained from MEC 1 (300 ml) at all applied voltage indicated no significant differences. Bio-HPR 190.76 cm^3, YH_2 76.16% and COD removal efficiency 57.6% are the lowest values were obtained in MEC1 (300 ml) at applied voltage 0.4 V. The values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) by *Escherichia coli* NRRL B-3008 from industrial wastewater in MEC 1 (300 ml), MEC 2 (400 ml) and MEC 3 (500 ml) with a variable applied voltage shown in Table (4).

The present results are in agreement with those reported by Baek et al. (2021) which concluded that applied voltage regulation in MEC has a direct effect on the activity of bacterial strains in oxidation and reduction reactions at the anode and cathode electrodes. Electrodes act as carriers for bacteria through biofilm formation as well as electron transfer. The applied voltage 0.8 V is the optimal voltage for bacterial activity on the anode surface and for the MEC to work efficiently.

Table 3. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by *Escherichia coli* NRRL B-3008 and domestic wastewater.

	MEC 1			MEC 2			MEC 3		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD_{in} (mg/L)	243	243	243	243	243	243	243	243	243
COD_{out} (mg/L)	64	61	57	47	46	46	43	42	40
COD removal efficiency (%)	73.66	74.89	76.54	80.65	81.06	81.06	82.3	82.71	83.53
$^nH_2$ (mol)	4.37	4.99	5.76	7.95	8.11	8.25	8.77	8.99	9.35
^{n}th (mol)	11.18	11.37	11.62	12.25	12.31	21.31	12.5	12.56	12.68
Bio-HPR (cm^3)	110.3	125.99	145.22	200.37	204.42	207.96	221.12	226.68	235.87
YH_2 %	39.11	43.94	49.56	64.9	65.88	67.03	70.19	71.6	73.76

Bio-HPR (LSD at 5% = 13.95), YH_2 % (LSD at 5% = 3.85).

Table 4. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by *Escherichia coli* NRRL B-3008 and industrial wastewater.

	MEC 1			MEC 2			MEC 3		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD_{in} (mg/L)	276	276	276	276	276	276	276	276	276
COD_{out} (mg/L)	117	109	101	73	71	67	52	44	33
COD removal efficiency (%)	57.6	60.5	63.40	73.55	74.27	75.72	81.15	84.05	88.04
$^nH_2$ (mol)	7.56	8.21	8.83	11.1	11.28	11.58	12.76	13.37	14.21
^{n}th (mol)	9.93	10.43	10.93	12.68	12.87	12.93	14	14.5	15.18
Bio-HPR (cm^3)	190.76	206.95	222.64	279.81	284.37	291.96	321.8	336.99	358.24
YH_2 %	76.16	78.68	80.77	87.53	87.64	89.54	91.2	92.22	93.64

Bio-HPR (LSD at 5% = 19.54), YH_2 % (LSD at 5% = 0.94).

3.3. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) by *Enterobacter aerogenes* DSM 30053

Enterobacter aerogenes has been used in many studies to produce bio-hydrogen, whether using MEC or dark fermentation. The hydrogen production process takes place under anaerobic conditions. *E. aerogenes* bacteria possess many distinctive characteristics that qualify them to produce bio-hydrogen. Firstly, it is facultative anaerobic bacteria, which makes it easier to deal with them in the biological processes of hydrogen production in various ways compared to obligate anaerobic bacteria. Secondly, they can grow using a wide range of carbon sources. Third, *E. aerogenes* bacteria use the pyruvate formate lyase (PFL) pathway to produce hydrogen by dark fermentation. Fourth, the optimal growth conditions are at pH 6-7 and temperature 38-40 ◦C (Ergal et al. 2018).

In MEC 1 (300 ml), MEC 2 (400 ml) and MEC 3 (500 ml) were used domestic wastewater as substrate for bio-hydrogen gas production by *Enterobacter aerogenes* DSM 30053. Significant differences were found between the highest values of Bio-HPR 316 cm^3, YH_2 91.17 % and COD removal efficiency 90.53 % were obtained in MEC 3 (500 ml) at applied voltage 0.4 V and other values were obtained in MEC2 (400 ml) at all applied voltage. In MEC 1 (300ml) were gave the lowest values of Bio-HPR 80.45 cm^3, YH_2 30.04 % and COD removal efficiency 69.95 % at applied voltage 0.8 V, which revealed significant negative relationship between the values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) at all applied voltage. Table (5).

Bio-HPR 348.1 cm^3, YH_2 92.86 % and COD removal efficiency 86.23 % are the highest values of were obtained in MEC 3 (500 ml) at applied voltage 0.4 V by industrial wastewater and *Enterobacter aerogenes* DSM 30053.

The values of Bio-HPR cm^3, YH_2 % and COD removal efficiency % were obtained in MEC 3 (500 ml) and other values were obtained in MEC2 (400 ml) at applied voltage 0.8 V revealed no significant differences were found between them. Bio-HPR 159.39 cm^3, YH_2 70.02 % and COD removal efficiency 52.17 % are the lowest values were obtained in MEC1 (300 ml) at applied voltage 0.8 V, which revealed significant negative relationship between values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) at all applied voltage in MEC1 (300 ml) Table 6.

These results were confirmed with Hasibar et al. (2020) which found that the *E. aerogenes* bacteria gave the highest of Bio-HPR using MEC at applied voltage of 0.8 V. He also used mixed bacterial cultures of *E. aerogenes* and *Clostridium acetobutylicum* to increase the Bio-HPR using the MEC and the dark fermentation method.

Table 5. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by *Enterobacter aerogenes* DSM 30053 and domestic wastewater.

	MEC 1			MEC 2			MEC 3		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD $_{in}$ (mg/L)	243	243	243	243	243	243	243	243	243
COD $_{out}$ (mg/L)	61	67	73	45	51	58	23	40	42
COD removal efficiency (%)	74.89	72.42	69.95	81.48	79.01	76.13	90.53	83.53	82.71
$^nH_2$ (mol)	5.48	4.29	3.19	8.39	7.20	5.94	12.53	9.29	8.95
^{n}th (mol)	11.37	11	10.62	12.37	12	11.56	13.75	12.68	12.56
Bio-HPR (cm^3)	138.13	108.28	80.45	211.5	181.65	149.77	316	234.27	225.67
YH_2 %	48.18	39.05	30.04	67.81	60.06	51.4	91.17	73.27	71.28

Bio-HPR (LSD at 5% = 16.13), YH_2 % (LSD at 5% = 4.58).

Table 6. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by *Enterobacter aerogenes* DSM 30053 and industrial wastewater.

	MEC 1			MEC 2			MEC 3		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD $_{in}$ (mg/L)	276	276	276	276	276	276	276	276	276
COD $_{out}$ (mg/L)	104	120	132	103	86	86	71	66	38
COD removal efficiency (%)	62.31	56.52	52.17	62.68	68.84	68.84	74.27	76.08	86.23
$^nH_2$ (mol)	8.55	7.26	6.32	8.61	9.95	11.46	11.18	11.61	13.81
^{n}th (mol)	10.75	9.75	9	10.81	11.87	13.06	12.81	13.12	14.87
Bio-HPR (cm^3)	215.55	183.17	159.39	217.07	250.97	288.92	281.84	292.8	348.1
YH_2 %	79.56	74.54	70.02	79.66	83.86	87.77	87.26	88.52	92.86

Bio-HPR (LSD at 5% = 20.96), YH_2 % (LSD at 5% = 2.53).

3.4. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) by *Pseudomonas aeruginosa* ATCC 27853

Bacteria are one of the important factors that affect the efficiency of MEC performance. Bacteria that have the ability to grow on the surface of the anode and form a biofilm are called electroactive bacteria. Domestic wastewater and industrial wastewater have been used as environments for the colonization of electroactive bacteria because these bacteria possess many terminal electron receptors that enable them to respire and grow. Electroactive bacteria can be isolated from different environments such as soil, rivers, wastewater and manure. These bacteria belong to different phyla, such as: Proteobacteria (*Pseudomonas, Geobacter* and *Shewanella*), Acidobacteria (*Geothrix*) and Firmicutes (*Clostridium*) (Cardeña et al. 2019 and Srivastava et al. 2023).

The results were obtained from using domestic wastewater in MEC1 (300 ml), MEC2 (400 ml) and MEC3 (500 ml) at all applied voltage for bio-hydrogen production by *Pseudomonas aeruginosa* ATCC 27853. Bio-HPR 268.08cm^3, YH_2 81.05 % and COD removal efficiency 86.41 % are the highest values were obtained in MEC3 (500 ml) at applied voltage 0.8 V, revealed to no significant differences were found between other values obtained from MEC3 (500 ml) at applied voltage 0.4 and 0.6 V.

Bio-HPR 146.23cm^3, YH_2 50.18 % and COD removal efficiency 76.13 % are the lowest values of were obtained in MEC1 (300 ml) at applied voltage 0.8 V which, indicated significant differences were found between other values were obtained in MEC2

(400 ml) and MEC3 (500 ml) at all applied voltage. Previously it is note that, positive relationship was found between the values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) and all applied voltage at MEC2 (400 ml) and MEC3 (500 ml). But significant negative relationship was found between all applied voltage and the values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) were obtained in MEC1 (300 ml) Table 7.

Results were achieved from using industrial wastewater as substrate for bio-hydrogen production by *Pseudomonas aeruginosa* ATCC 27853 in MEC1 (300 ml), MEC2 (400 ml) and MEC3 (500 ml) at all applied voltage. Significant differences were found between the highest values of Bio-HPR 343.57cm^3, YH_2 92.87 % and COD removal efficiency 85.14 % were obtained in MEC3 (500 ml) at applied voltage 0.8 V, which revealed significant positive relationship between this values and all applied voltage at MEC2 (400 ml) and MEC3 (500 ml). In MEC1 (300 ml) at applied voltage 0.8 V were obtained the lowest values of Bio-HPR 219.6 cm^3, YH_2 80.32 % and COD removal efficiency 62.68 % this results were revealed to significant negative relationship between applied voltage and values of Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) Table 8

These results are also consistent with Wei et al. (2022) which studied variety of microbial species in MEC for organic matter decomposition and biofilm formation under anaerobic conditions. He also studied the effect of different values in applied voltage on the efficiency of MEC in producing bio-hydrogen. MEC has been used as a biological technology to treat wastewater and remove bromoaniline.

Table 7. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by *Pseudomonas aeruginosa* ATCC 27853 and domestic wastewater.

	MEC 1			**MEC 2**			**MEC 3**		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD_{in} (mg/L)	243	243	243	243	243	243	243	243	243
COD_{out} (mg/L)	55	57	58	51	50	48	40	34	33
COD removal efficiency (%)	77.36	76.54	76.13	79.01	79.42	80.24	83.53	86.01	86.41
$^nH_2$ (mol)	6.34	6.02	5.80	7.12	7.38	7.69	9.23	10.40	10.63
^{n}th (mol)	11.75	11.62	11.56	12	12.06	12.18	12.68	13.06	13.12
Bio-HPR (cm^3)	159.89	151.8	146.23	179.63	186.2	193.79	232.76	262.1	268.08
YH_2 %	53.99	51.81	50.18	59.4	61.25	63.1	72.79	79.62	81.05

Bio-HPR (LSD at 5% = 13.53), YH_2 % (LSD at 5% = 3.43).

Table 8. Bio-HPR (cm^3), YH_2 (%) and COD removal efficiency (%) in MECs by *Pseudomonas aeruginosa* ATCC 27853 and industrial wastewater.

	MEC 1			**MEC 2**			**MEC 3**		
	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V	0.4V	0.6V	0.8V
COD_{in} (mg/L)	276	276	276	276	276	276	276	276	276
COD_{out} (mg/L)	101	101	103	87	85	82	66	48	41
COD removal efficiency (%)	63.4	63.4	62.68	68.47	69.2	70.28	76.08	82.6	85.14
$^nH_2$ (mol)	9.15	8.89	8.71	9.95	10.11	10.44	11.60	13.07	13.63
^{n}th (mol)	10.93	10.93	10.87	11.75	11.87	12.18	13.12	14.25	14.68
Bio-HPR (cm^3)	230.73	224.15	219.6	250.79	255.02	263.12	292.46	329.4	343.57
YH_2 %	83.71	81.32	80.32	84.68	85.21	86.14	88.41	91.72	92.87

Bio-HPR (LSD at 5% = 10.68), YH_2 % (LSD at 5% = 0.81).

Conclusions

The COD removal efficiency (%) determines the efficiency of MEC performance in bio-hydrogen production and wastewater treatment. The values of Bio-HPR (cm^3), *Y*H2 (%), and COD removal efficiency (%) obtained from MEC depend on many factors, including the substrate, bacterial strain, and applied voltage. Hydrogen production rates Bio-HPR (cm^3) are directly proportional to the COD removal efficiency (%), as every mole of COD was removed produces 2 moles of hydrogen. We must work to develop and improve efficiency of MEC. The MEC has been studied by many researchers in the fields of energy production and water treatment. MEC has an effective role in producing bio-hydrogen and treating wastewater, which reflects on confronting climate change.

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