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Applications of *Escherichia coli* and *Enterobacter aerogenes* as Electroactive Bacteria for Bio-Hydrogen Production in Microbial Electrolysis Cells

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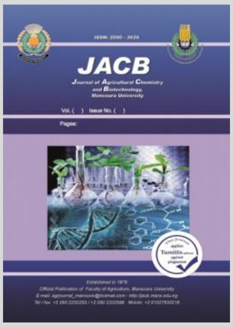

ABSTRACT

Green technology for bio-hydrogen production refers to the use of electrochemical systems and electroactive bacteria. Microbial electrolysis cells (MECs) are one of these systems. Among the electroactive bacteria were used in this study *Escherichia coli* NRRL B-3008 and *Enterobacter aerogenes* DSM 30053. Water from Al-Salam Canal was also used as a substrate according to chemical structure (H_2O) of water as cheap source to produce hydrogen gas by using microorganisms *i.e* bacteria. The result obtained were indicate that *Escherichia coli* NRRL B-3008 gave the highest values of bio-hydrogen production rates (Bio-HPR = 102.7 cm^3) and hydrogen yield (YH_2 = 57.19 %) was obtained from MEC (anode 500ml) and an applied voltage 0.8V. *Enterobacter aerogenes* DSM 30053 gave the highest values of Bio-HPR = 132.57 cm^3 and YH_2 = 70.13 % was obtained from MEC (anode 500ml) and an applied voltage 0.4V. While, the highest volume of Bio-H_2 without bacteria was 51.1 cm^3 and YH_2 = 31.18 % with an applied voltage 0.8 V (anode 500ml). Therefore, the bacterial electrolysis are very important biological process for highest hydrogen yield by bio-hydrogen production.

Keywords: Electroactive bacteria, Bio-hydrogen production rates (Bio-HPR), Hydrogen yield (YH_2 %).

INTRODUCTION

Biofuel, also called bioenergy, includes bioethanol, biogas, bio-hydrogen, biodiesel, and methanol. The term biofuel refers to fuel that is produced from various biological processes by the action of microorganisms on organic waste and biomass. Biofuel is produced in special reactors called bioreactors, electrochemical systems or various biological fermentation methods. Biofuels are considered highly efficient renewable energy that can be used as alternatives in the future to replace fossil fuels. Hydrogen is considered one of the most important types of biofuel. For example, when comparing hydrogen to methane, we find that hydrogen has a combustion rate of about 142 MJ kg^{-1} compared to methane 55MJ kg^{-1}. The product of hydrogen gas combustion is water vapor and is therefore an environmentally friendly fuel (Gumilar *et al.,* 2019). The biological technology of bio-hydrogen production cells is known as electro-fermentation, bio-catalysed electrolysis cells (BECs) or microbial electrolysis cells (MECs), and it is within bio-electrochemical systems (Parvanova-Mancheva *et al.,* 2022).The hydrogen production process in MEC takes place through the anode chamber (reaction chamber). The anode chamber contains the substrates, microorganisms and anode electrode. Bacteria work to decompose the organic matter present in the substrate and produce protons, electrons, and carbon dioxide. Bacteria also form a biofilm on the surface of anode electrode to stimulate the transfer of electrons through an applied electrical voltage of about (0.1 - 0.9 V) to the cathode chamber via a connecting wire between the anode electrode and the cathode electrode. The cathode chamber is filled with one of the following different solutions (phosphate solution, bicarbonate buffers, salt solutions or distilled water). The anode chamber and the cathode chamber are separated by ion exchange membranes (IEM) or cation exchange membranes (CEM) or Salt Bridge. These membranes transfer protons from the anode chamber to the cathode chamber to combine with electrons to form hydrogen gas (Osman *et al.,* 2020). Electroactive microorganisms are those that can generate an electric current and transfer electrons directly without the need to add intermediaries or use electron shuttles. Electroactive microorganisms are the focus of work in modern bio-electrochemical systems. These systems include microbial fuel cells (MFCs) that are used to produce electricity, as well as wastewater treatment and detection of toxic chemicals, and microbial electrolysis cells (MECs) that are used to produce bio-hydrogen and bio-methane. Also other microbial electrochemical techniques (METs) that can be used in drinking water desalination and hydrogen peroxide production processes (Lovley and Holmes 2022). Microorganisms transfer electrons in direct ways, such as by direct contact with outer membrane cytochromes located on the cell surface or on conductive extensions. Or indirectly using mediators such as flavins that can transfer electrons between the cell and the anode electrode (Reguera, 2018). There are several strains from bacteria called electrochemically active bacteria, these 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).

This research focuses on the application of using two strains of electroactive bacteria to produce bio-hydrogen: of *Escherichia coli* NRRL B-3008 and *Enterobacter aerogenes* DSM 30053. The MEC using as a bioreactor to produce

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hydrogen from Al-Salam Canal water as a substrate. Application of three different volumes of MEC (anode 300, 400, and 500 ml) at variable applied voltage (0.4, 0.6 and 0.8V). Measurement the bio-hydrogen production rates according to the variables of anode volume and electrical applied voltage. Evaluation of hydrogen yield rates according to chemical changes in the substrate such as chemical oxygen demand (COD).

MATERIALS AND METHODS

Strains

Two strains of electroactive bacteria were used in this research to produce bio-hydrogen by MECs: *Escherichia coli* NRRL B-3008 and *Enterobacter aerogenes* DSM 30053. Strains were obtained from Ain Shams Univ. (MIRCEN – Faculty of Agriculture), Cairo, Egypt. It is prepared using Nutrient broth medium (13gm /1 L distilled water) according to Afify *et al.,* (2023a).

Substrate

Samples of Al-Salam Canal water were used to produce bio-hydrogen, which were obtained from the Baloza area (water-lifting No.5), North Sinai Governorate, Egypt. Water samples were analyzed in the central laboratory - Desert Research Center and found to contain: Organic matter (7.3 %), Chemical oxygen demand (148 mg /L), Total dissolved solids (195 mg /L), biochemical oxygen demand (235 mg /L), turbidity (0.92 NTU), pH = 7.6 and electrical conductivity (3.05 ms). HCl acid (1M) was used to adjust the pH to 7. A buffer solution of sodium phosphate (0.2 M) is also used to maintain the pH (Abd-Elrahman *et al.,* 2022b).

MEC

Three different types of LMEC were used according to the volume of the anode chamber (300, 400, 500 ml). But they have the same composition and method of operation. Figure (1)

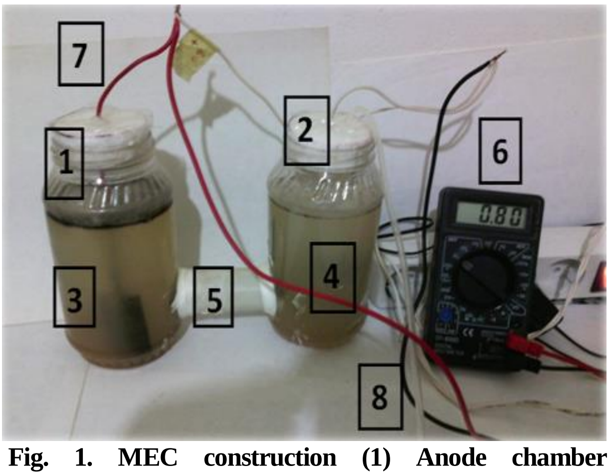

Fig. 1. MEC construction (1) Anode chamber (2) Cathode chamber (3) Anode electrode (4) Cathode electrode (5) Salt bridge (6) Applied voltage (7)Copper wire (8) Tube hydrogen exit.

Shows the structure of MEC as follows: 1 - The anode chamber, which contains the anode electrode, the substrate and the bacterial strain. The anode chamber is considered the reaction center in MEC, where bacteria able to decompose the organic matter present in the substrate for the electron, proton, and carbon dioxide are produced. 2 - The cathode chamber contains cathode electrode and distilled water. It is where hydrogen gas is produced. 3 - Anode electrode, where carbon brush was used as a positive electrode in the anode chamber. 4 - The cathode electrode, which stainless steel was used as a negative electrode. 5 - The salt bridge, which was used as an alternative to the proton exchange membrane. The salt bridge transfers the proton resulting from the decomposition of organic matter by bacteria in the anode chamber to the cathode chamber. It was prepared using potassium chloride salt (KCl) and agar. 6 – Applied voltage (Power supply): 0.4, 0.6, and 0.8 V were used as regular voltage to operate the MEC during the experiment. 7 - Copper wire: The wire was used to connect between the anode electrode, the cathode electrode, and the electrical voltage source. The copper wire transfers electrons from the anode chamber to the cathode chamber. 8- The hydrogen exit tube, which is located at the top of the cathode chamber and was used to collect the hydrogen produced from the MEC.

Calculate the Bio-HPR (cm^3) and Hydrogen yield (YH_2 %)

The water displacement method was used to estimate bio-hydrogen production rates using the following equation: Afify *et al.,* (2017b)

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

Where:

(π) = 3.14 and (r) = Radius of the burette tube.

Hydrogen yield (YH_2 %)

Before calculating the value of Hydrogen Yield (YH_2), the values of ($^nH_2$) and (^{n}th) must be calculated first. $^nH_2$ is the number of moles of hydrogen produced. It was calculated by following equation:

$$^n\mathbf{H_2} = \frac{\mathbf{Bio, HPR}}{\mathbf{R\,T}}$$

Where:

R = gas constant = 0.08314 L bar / K mol and (T) =303 K is the absolute temperature.

^{n}th is the number of moles in the substrate that were converted during the reaction in the anode chamber. It was calculated by the equation:

$$^n\mathbf{th} = \frac{\mathbf{2(COD_{in} - COD_{out})}}{\boldsymbol{MO_2}}$$

Where:

MO_2 =32 gm/mol (the molecular weight of oxygen). COD $_{in}$ is the concentration of chemical oxygen demand 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.

Therefore, the Hydrogen Yield (YH_2) is calculated by the equation: Logan *et al.* (2008).

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

Statistical analysis

The statistical analysis program (Statistix 9) was used to compare and find significant differences between the results obtained by finding the value of the LSD.

RESULTS AND DISCUSSION

Control experiment

A control experiment was conducted in this research without using bacterial strains to produce bio-hydrogen, in order to clarify the effective role it plays in decomposing the organic matter present in the substrate. Table (1) shows the values of Bio-HPR (cm^3) from Al-Salam Canal water through three different volumes of MEC (anode 300, 400, and 500 ml), where a regular electrical voltage was applied. The results obtained show that there are differences in the beginnings of hydrogen gas production.

The first production of hydrogen gas was started on the 6th day at an applied voltage 0.6 and 0.8 V, then on the 7th day at an applied voltage 0.4 V using MEC (anode 500 ml) until the 10th day at an applied voltage 0.4 and 0.6 V by MEC (anode 300 ml). The results obtained indicate that the highest values of Bio-HPR (51.1 cm^3) was obtained from using MEC (anode 500ml) at an applied voltage 0.8 V. While the lowest values of Bio-HPR (19.73cm^3) was obtained using MEC (anode 300 ml) at an applied voltage 0.4 V. That is, the values of Bio-HPR were directly proportional to the volumes of the anode chamber and applied voltage.

Table 1. Values of Bio-HPR (cm^3) from Al-Salam Canal water in MEC.

MECs	Anode 300 ml			Anode 400 ml			Anode 500 ml		
Voltage	0.4	0.6	0.8	0.4	0.6	0.8	0.4	0.6	0.8
Day	Bio-HPR(cm^3)/ Day								
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.06	6.07
7	0.0	0.0	0.0	0.0	6.07	8.6	6.57	11.13	13.66
8	0.0	0.0	0.0	5.06	12.14	16.19	12.65	17.2	19.73
9	0.0	0.0	8.6	11.13	19.22	23.78	18.72	23.27	26.81
10	7.08	8.09	13.15	17.2	26.31	29.85	24.79	29.34	33.9
11	13.15	14.16	17.71	24.28	32.38	33.9	30.86	35.42	39.97
12	16.69	17.2	20.74	33.39	34.91	37.95	36.93	41.49	46.04
13	18.72	20.24	22.77	35.92	36.93	40.98	39.97	44.52	49.08
14	19.73	21.75	23.78	36.93	38.96	43.01	41.49	46.04	50.6
15	19.73	22.26	24.28	37.44	39.46	44.02	41.49	46.55	51.1
Bio-HPR (cm^3)	19.73	22.26	24.28	37.44	39.46	44.02	41.49	46.55	51.1
LSD at 5%		5.45			4.55			3.96	

Table (2) shows the different values of ($^nH_2$) and (^{n}th), where values of ($^nH_2$) indicate the number of moles of hydrogen produced, while values of (^{n}th) indicate the number of moles in the substrate that were converted during the reaction in the anode chamber. The values of ($^nH_2$) were ranged between 0.78 – 2.02mol, while the values of (^{n}th) were ranged between 5.56 – 6.5mol.

Different values of hydrogen yield (YH_2 %) were calculated from the values of ($^nH_2$) and (^{n}th). The results obtained indicate that the highest value of YH_2 = 31.18 % was obtained from MEC (anode 500ml) and an applied voltage 0.8V, which indicating the significant differences were found between other values of YH_2%. While the lowest value of YH_2 = 14.05 % was obtained from MEC (anode 300ml) and an applied voltage 0.4V. No significant differences were found between all values of YH_2% and values of Bio-HPR (cm^3) at MEC (anode 300ml) with all applied voltage. Also, the values of YH_2% and values of Bio-HPR (cm^3) were directly proportional to the volumes of the anode chamber and applied voltage. The YH_2% and Bio-HPR (cm^3) from AL-Salam canal water revealed to significant positive relationship between them.

These results agree with Heidrich *et al.*, (2014) who obtained the highest rates of bio-hydrogen production from wastewater of 0.015 L/L/day from using MEC in the operating temperature range of 13-22°C.

Table 2. Values of (YH_2 %) from Al-Salam Canal water in MEC

MECs	Anode 300 ml			Anode 400 ml			Anode 500 ml			LSD at 5%
Voltage	0.4	0.6	0.8	0.4	0.6	0.8	0.4	0.6	0.8	
COD_{in} (mg/L)	148	148	148	148	148	148	148	148	148	
COD_{out} (mg/L)	50	49	49	47	47	46	47	46	44	
$^nH_2$ (mol)	0.78	0.88	0.96	1.48	1.56	1.74	1.64	1.84	2.02	
^{n}th (mol)	5.56	6.18	6.18	6.31	6.31	6.37	6.31	6.37	6.5	
Bio-HPR (cm^3)	19.73	22.26	24.28	37.44	39.46	44.02	41.49	46.55	51.1	3.48
YH_2 %	14.05	14.27	15.56	23.52	24.79	27.38	26.07	28.97	31.18	1.9

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

The effect of the *Escherichia coli* NRRL B-3008 on the beginning of hydrogen gas production is evident in the results shown in Table (3) compared to the control experiment. Hydrogen gas was started to be produced on the 5th day in both the MEC (anode 500 ml) at an applied voltage 0.8V and the MEC (anode 400 ml) at an applied voltage 0.6 and 0.8V, then on the 6th day in both the MEC (anode 400ml) at an applied voltage 0.4V and MEC (anode 300 ml) at an applied voltage 0.8V, And finally on the 7th day in MEC (anode 300 ml) at an applied voltage 0.4 and 0.6V. The lowest value of Bio-HPR = 44.52 cm^3 was obtained using MEC (anode 300 ml) at an applied voltage 0.4 V. While the highest value of Bio-HPR = 102.7 cm^3 was obtained from using MEC (anode 500ml) at an applied voltage 0.8 V. That is, the values of Bio-HPR (cm^3) were directly proportional to *Escherichia coli* NRRL B-3008, volumes of the anode chamber and applied voltage.

The results were obtained from using *Escherichia coli* NRRL B-3008 to produce bio-hydrogen indicate that there are significant differences between the highest value of (YH_2 %) and the lowest value of (YH_2 %). The value of (YH_2 %) was directly proportional to Bio-HPR (cm^3). The results also indicate that the highest value of YH_2 = 57.19 % was obtained from MEC (anode 500ml) and an applied voltage 0.8V. While the lowest value of YH_2 = 26.91 % was obtained from MEC (anode 300ml) and an applied voltage 0.4V. All results were obtained shown in Table 4. These results are consistent with Poladyan *et al.*, (2020) who produced bio-hydrogen from *Escherichia coli* from biomass of paper and cardboard waste by biological methods using dark fermentation processes.

Table 3. Values of Bio-HPR (cm^3) by *Escherichia coli* NRRL B-3008 in MEC

MECs	Anode 300 ml			Anode 400 ml			Anode 500 ml		
Voltage	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**
Day					Bio-HPR(cm^3)/ Day				
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	8.6	11.13	0.0	0.0	6.91
6	0.0	0.0	11.13	7.59	14.67	23.27	8.6	7.59	22.26
7	7.08	9.61	18.21	13.66	25.8	37.44	17.71	19.73	36.43
8	11.63	20.74	27.32	29.85	37.95	51.61	33.9	35.92	48.07
9	22.77	31.87	38.96	44.02	52.11	64.26	51.1	49.08	64.26
10	34.4	39.97	50.09	56.67	64.26	75.39	67.29	62.23	78.43
11	38.96	46.04	56.16	68.81	73.37	83.49	75.39	78.43	90.57
12	40.98	50.09	60.21	74.88	81.46	87.53	79.44	86.52	96.64
13	43.51	52.62	62.23	76.91	85.51	90.06	81.46	90.57	99.68
14	44.52	53.63	64.26	78.43	87.53	91.58	82.47	92.59	102.7
15	44.52	54.14	65.27	78.93	88.55	92.09	82.98	93.61	102.7
Bio-HPR (cm^3)	44.52	54.14	65.27	78.93	88.55	92.09	82.98	93.61	102.7
LSD at 5%		5.91			4.58			3.94	

Table 4. Values of (YH_2 %) by *Escherichia coli* NRRL B-3008 in MEC

MECs	Anode 300 ml			Anode 400 ml			Anode 500 ml			LSD at 5%
Voltage	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**	
COD $_{in}$ (mg/L)	148	148	148	148	148	148	148	148	148	
COD $_{out}$ (mg/L)	43	42	40	39	37	36	38	36	34	
$^nH_2$ (mol)	1.76	2.14	2.59	3.13	3.51	3.65	3.29	3.71	4.07	
^{n}th (mol)	6.56	6.62	6.75	6.81	6.93	7	6.87	7	7.12	
Bio-HPR (cm^3)	44.52	54.14	65.27	78.93	88.55	92.09	82.98	93.61	102.7	6.46
YH_2 %	26.91	32.42	38.37	45.97	50.64	52.2	47.88	53.05	57.19	3.36

***Enterobacter aerogenes* DSM 30053**

Among the electroactive bacteria are *Enterobacter aerogenes* and *Bacillus subtilis*, which can produce electric current and transfer electrons in pure environments. However, the presence of these bacteria in mixed environments increases the production of electrical current further as a result of increasing their ability to transfer electrons using external electron acceptors on the cell surface (Doyle and Marsili 2018).

On the 3rd day, hydrogen gas was produced in MEC (anode 400 ml) at an applied voltage 0.4V and in MEC (anode 500 ml) at an applied voltage 0.4 and 0.8V. In MEC (anode 300 ml) hydrogen gas was produced on 6th day at an applied voltage 0.4V. Hydrogen gas was produced continued for 15th days in all MEC (anode 300, 400 and 500 ml) at all applied voltage. Table (5) shows the effect of *Enterobacter aerogenes* DSM 30053 on hydrogen production rates from Al-Salam Canal water using MEC. The results were obtained indicate that there are significant differences between the highest value of Bio-HPR = 132.57 cm^3 in MEC (anode 500 ml) at an applied voltage 0.4V and the value of Bio-HPR = 112.83 cm^3 at an applied voltage 0.8V in the same anode. The lowest value of Bio-HPR = 39.97 cm^3 was obtained in MEC (anode 300 ml) at an applied voltage 0.8V. Which indicates that there are significant differences with value of Bio-HPR = 66.79 cm^3 at an applied voltage 0.8V in the same anode. The results indicate a negative relationship between Bio-HPR and applied voltage. The values of (^{n}th) were ranged between 6.43 – 7.68mol, which are calculated according to different values of Bio-HPR (cm^3) and gas constant (R = 0.08314 L bar / K mol) at absolute temperature. The different values of ($^nH_2$) were ranged between 1.58–5.26mol, which is calculated from changes in COD $_{out}$ values. Thus, different values of (YH_2 %) were obtained, which are explained in Table 6.

Table 5. Values of Bio-HPR (cm^3) by *Enterobacter aerogenes* DSM 30053 in MEC

MECs	Anode 300 ml			Anode 400 ml			Anode 500 ml		
Voltage	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**
Day					**Bio-HPR(cm^3)/ Day**				
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	11.13	0.0	0.0	12.14	0.0	8.09
4	0.0	0.0	0.0	24.28	0.0	0.0	23.27	6.07	17.2
5	0.0	0.0	0.0	32.89	9.1	6.57	25.92	17.2	29.34
6	6.72	0.0	0.0	49.08	21.25	11.13	51.61	34.91	39.97
7	12.65	6.07	5.56	57.17	33.39	23.27	65.27	47.56	52.11
8	23.27	16.19	12.65	66.28	45.54	35.92	82.47	60.21	64.26
9	34.9	27.32	18.72	75.39	54.64	48.07	94.62	73.37	79.94
10	47.56	39.46	25.8	83.49	64.26	57.17	107.27	87.53	88.55
11	55.15	41.49	32.89	90	73.37	66.28	116.38	98.67	97.65
12	61.73	45.54	36.43	96.64	79.94	74.38	125.48	107.27	104.74
13	64.76	47.56	38.45	100.69	86.02	77.92	129.53	115.36	108.79
14	66.28	48.57	39.97	102.71	89.05	80.96	131.56	118.4	111.82
15	66.79	48.57	39.97	103.73	90.57	82.47	132.57	119.92	112.83
Bio-HPR (cm^3)	66.79	48.57	39.97	103.73	90.57	82.47	132.57	119.92	112.83
LSD at 5%		3.35			4.25			6.29	

Table 6. Values of (YH_2 %) by *Enterobacter aerogenes* DSM 30053 in MEC

MECs	Anode 300 ml			Anode 400 ml			Anode 500 ml			LSD at 5%
Voltage	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**	**0.4**	**0.6**	**0.8**	
COD $_{in}$ (mg/L)	148	148	148	148	148	148	148	148	148	
COD $_{out}$ (mg/L)	40	43	45	34	36	37	28	31	30	
$^nH_2$ (mol)	2.65	1.92	1.58	4.11	3.59	3.27	5.26	4.75	4.47	
^{n}th (mol)	6.75	6.56	6.43	7.12	7	6.93	7.5	7.68	7.62	
Bio-HPR (cm^3)	66.79	48.57	39.97	103.73	90.57	82.47	132.57	119.92	112.83	10.28
YH_2 %	39.25	29.36	24.63	57.76	51.34	47.16	70.13	61.89	58.71	5.05

The highest value of YH_2 = 70.13 % was obtained from MEC (anode 500ml) and an applied voltage 0.4V, which revealed significant differences with the highest value of Bio-HPR = 132.57 cm^3 . While the lowest value of YH_2 = 24.63 % was obtained in MEC (anode 300 ml) at an applied voltage 0.8V.The values of (YH_2 %) are directly proportional to the volume of anode chamber and inversely proportional to the applied voltage. These results are consistent with Hasibar *et al.*, (2020) who used both *Enterobacter aerogenes* and *Clostridium acetobutylicum* as mixed bacterial cultures in MEC to produce bio-hydrogen at an applied voltage of 0.8 V. The authors confirmed that the use of microorganisms increases hydrogen production, especially substrates that contain a high percentage of organic matter. The use of mixed bacterial cultures and the applied voltage in the MEC led to a maximum of hydrogen reaching about 0.93mmol L^{-1} h^{-1} for the mixed bacterial cultures at an applied voltage 0.8 V.

CONCLUSION

All scientists and researchers specialized in bio-hydrogen production research agree that it is an effective and alternative solution to fossil fuels. Hydrogen is the solution to confront environmental pollution and a source of clean energy to confront the climate changes facing the whole world. In conclusion, microbial electrolysis cells are considered one of the best biological methods used to produce bio-hydrogen. The efficiency of MEC in hydrogen production reaches 91%. The efficiency of MEC is determined by several factors, including the substrate and microorganisms, in addition to factors specific to composition and operation.

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تطبيقات الـ *Escherichia coli* NRRL B-3008 و الـ *Enterobacter aerogenes* DSM 30053 كبكتيريا نشطة كهربياً لإنتاج الهيدروجين الحيوى في خلايا التحليل الكهربائي الميكروبية

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الملخص

تشير التكنولوجيا الخضراء لإنتاج الهيدروجين الحيوي إلى إستخدام الأنظمة الكهروكيميائية والبكتيريا النشطة كهربياً. تعتبر خلايا التحليل الكهربائي الميكروبية (MECs) واحدة من هذه الأنظمة. ومن بين البكتيريا النشطة كهربياً التى تم إستخدامها في هذه الدراسة *Escherichia coli* NRRL B-3008 و*Enterobacter aerogenes* DSM 30053 . كما تم إستخدام مياه ترعة السلام كمادة لإنتاج الهيدروجين طبقا لتركيبها الكيميائى الذى يتضمن ذرتين من الهيدروجين وذرة أكسجين من أجل الحصول على الهيدروجين من مصادر بسيطة بل سهلة ورخيصة . تشير النتائج إلى أن بكتيريا *Escherichia coli* NRRL B-3008 أعطت أعلى قيم لمعدلات إنتاج الهيدروجين الحيوى ١٠٢,٧ سم^3 ونسبة محصول الهيدروجين ٥٧,١٩ % تم الحصول عليها من الـ MEC (الأنود ٥٠٠ مل) عند جهد كهربي ٠,٨ فولت. أعطت بكتيريا *Enterobacter aerogenes* DSM 30053 أعلى قيم من معدلات الهيدروجين الحيوى ١٣٢,٥٧ سم٣ و نسبة محصول الهيدروجين ٧٠,١٣ % تم الحصول عليها من الـ MEC (أنود ٥٠٠ مل) عند جهد كهربى ٠,٤ فولت. بينما تم الحصول على أعلى قيمة إنتاج الهيدروجين الحيوى ٥١,١ سم^{3}ونسبة محصول الهيدروجين ٣١,١٨ % من مياة هذه الترعة دون إضافة البكتيريا. ولهذا تعتبر خلايا التحليل الكهربائى الميكروبية من العمليات البيولوجية الهامة لإعطاء محصول هيدروجين عالى عند إنتاج الهيدروجين الحيوى .