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Effect of Power Supply and Bacteria on Bio-hydrogen Production Using Microbial Electrolysis Cells (MECs)

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ABSTRACT

Power supply and bacteria were very important factors effecting on Bio-hydrogen production. In this study 0.4 V, 0.6 V and 0.8 V were used as power supply for Bio-hydrogen production in microbial electrolysis (MECs) by *Enterobacter aerogenes* DSM 30053 and without addition bacteria. Industrial wastewater was used substrate. The highest hydrogen yield 92.86 % and the highest volume of Bio-H_2 348.1 cm^3 production by *Enterobacter aerogenes* DSM 30053 with power supply 0.8 V at the anode chamber 500 ml. While the highest hydrogen yield 71.94 % and the highest volume of Bio-H_2 production 165.46 cm^3 without addition of bacteria at the same condition

Keywords: Power supply, Microbial electrolysis cells, Bio-hydrogen, *Enterobacter aerogenes* DSM 30053, Industrial wastewater.

INTRODUCTION

Many factors effecting on bio-hydrogen production and MEC performance, such as bacteria, power supply, electrons transfer mechanism, substrates, solution electrical conductivity, pH, electrode (anode and cathode) materials, physical and chemical characteristic of electrodes and separator or membrane. MEC is needed power supply to obtain energy for bio-hydrogen production, power supply affect on bio-hydrogen, yield of hydrogen, growth of bacteria, electron transfer and efficiency of anode electrode. MECs were operated at power supply 0.3 –1 V. Power supply (1.14 V) are not suggested on the grounds that the electrical vitality information is so substantial and MEC turns out to be more like a water electrolysis prepare. Power supply lower than 0.3 V may result in low bio-hydrogen production rate and MECs system performance Liu *et al*., (2010) .

Bacteria can be transfered electron from the substrate or wastewater which contains organic matter from anode to cathode electrodes without mediators. Various types of bacteria (*Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Clostridium butyricum, Geobacter sulfurruducens and Shewanella putrefaciens*) are capable of electrons accepting and production bio-hydrogen under anaerobic conditions at the negative chamber in MEC Cucu *et al*., (2013).

This study aims to studies the effect of with power supply (0.4 V, 0.6 V and 0.8 V) and *Enterobacter aerogenes* DSM 30053 in MEC at the anode chamber 300, 400 and 500 ml for hydrogen yield (%) and Bio-hydrogen production from industrial wastewater.

MATERIALS AND METHODS

Preparation of Bacteria and Substrate:

Enterobacter aerogenes DSM 30053 was used as bacteria for Bio-hydrogen. It was obtained from microbiological resource center (Cairo MIRCEN), Fac. of Agric., Ain Shams Univ., Cairo, Egypt. Nutrient broth medium (13gm / liter of distilled water) was used for preparation of bacterial culture.

Industrial wastewater were obtained from Pickles Factory – small industrial area, EL-Obour city, EL-Qaliubiya, Egypt. Drops of 1M Na OH (4 gm Na OH / 100 ml distilled water) was added to the substrate to bring the pH up to 7. After adjusted the required pH added 0.2M sodium phosphate buffer solution (Abd El Rahman, 2017).

Microbial electrolysis cells (MECs):

MEC was used as Bio-electrochemical reactor for Bio-hydrogen production. It consists of anode and cathode chambers separated by salt bridge (agar 20% + 1 M of Potassium Chloride) as membrane. Volume of each chamber has a 300, 400 and 500 ml. Carbon brush (No.34 D) plate as anode electrode and stainless steel (304) as cathode electrode were connected to power supply. Anode chamber was filled with industrial wastewater (300, 400 and 500 ml) and 30, 40 and 50 ml (10 % v/v) of bacterial culture. Cathode chamber was filed by 300, 400 and 500 ml of distilled water. Copper wire connected between positive and negative electrodes of power supply (0.4 V, 0.6 V and 0.8 V / 500 mA / DC / 50 Hz) (Abd El Rahman, 2017).

Volume of Bio-hydrogen (Bio-H_2 cm^3):

Bio-hydrogen produced in cathode chamber was collected in burettes tubes by downward displacement of water Ujwal *et al.,* (2015).

$$\text{Volume of Bio-}H_2\ (cm^3) = \text{length of burette reading (cm)} \times \pi r^2\ (cm^2)$$

Where: $\pi = 3.14$, r = radius of burette tube

Hydrogen yield (YH_2 %):

Hydrogen Yield (YH_2) is the amount of hydrogen production from a substrate. It is calculated as: Logan *et al.,* (2008)

$$YH_2 = (^{n}H_2 / {}^{n}th) \times 100\ \%$$

Where: $^{n}H_2$ is the moles of hydrogen were produced in the experiments is calculated as:

$$^{n}H_2 = VH_2 / R\,T$$

VH_2 is volume of Bio-H_2 ,(R) is gas constant (0.08314 L bar / K mol) and (T) is the absolute temperature (303 K).

^{n}th is the moles of substrate converted. The hydrogen yield was based on COD is calculated each mole of COD removed could produce 2 mol of hydrogen. ^{n}th was calculated as:

$$^{n}th = 2\,\Delta COD\ /\ M\,O_2$$

$$\Delta COD = COD_i - COD_e$$

Where: $M\,O_2$ (32 gm / mol) is the molecular weight of oxygen, (COD_i) is the COD concentration of the substrate at the beginning and (COD_e) is the COD concentration of the substrate at the end.

Statistical analysis:

Statistical analysis of data was carried out according to (Statistix 9) for Windows using LSD test to compare between means values.

RESULTS AND DISCUSSION

Effect of power supply for Bio-hydrogen and Hydrogen yield (%) produced from industrial wastewater without bacteria:

Power supply 0.4 V, 0.6 and 0.8 V were used for electron transfer from anode to cathode chamber in MECs (300, 400 and 500 ml) for Bio-hydrogen production from industrial waste water. 300, 400 and 500 ml of industrial wastewater were filled in anode chamber and 300, 400 and 500 ml of distilled water in cathode chamber respectively. With power supply 0.4 V the hydrogen gas production in cathode chamber started from sixth day at anode chamber 300 ml of industrial wastewater and fourth day with power supply 0.8 V at anode chamber 500 ml. Significant differences were found between the highest volume of Bio-H_2 165.46 cm^3 with power supply 0.8 V at the anode chamber 500 ml and the lowest volume of Bio-H_2 79.44 cm^3 was obtained with power supply 0.4 V at anode chamber 300 ml (Table 1).

A variable of power supply were able to bio-hydrogen production in MECs at the anode chamber 300, 400 and 500 ml from industrial wastewater with, which indicates increasing industrial wastewater degradation rat.

Industrial wastewater was reported to be good substrate for bio-hydrogen production in MECs. The experiments carried out by Ujwal *et al.*, (2015) they also used the wastewater from sugar industry for bio-hydrogen production in MECs without bacteria.

Table 1. Effect of power supply 0.4 V, 0.6 V and 0.8 V for Bio-H_2 (cm^3) production at anode chamber 300, 400 and 500 ml from industrial wastewater without bacteria.

Days	0.4 V for Bio-H_2 produced (cm^3) at anode chamber			0.6 V for Bio-H_2 produced (cm^3) at anode chamber			0.8 V for Bio-H_2 produced (cm^3) at anode chamber		
	300 ml	400 ml	500 ml	300 ml	400 ml	500 ml	300 ml	400 ml	500 ml
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	8.6	0.0	13.15	11.13	0.0	12.14	12.14
5	0.0	11.63	26.31	8.6	29.34	28.33	11.13	30.36	29.85
6	7.59	24.79	45.03	21.25	44.02	46.55	24.79	48.07	48.57
7	19.73	38.96	64.26	33.39	58.69	65.78	35.92	64.26	68.31
8	32.38	51.1	81.97	46.55	72.86	85.51	47.56	78.43	89.56
9	45.03	64.26	96.14	57.17	85	103.22	58.69	92.59	110.81
10	56.16	76.91	110.3	65.78	96.64	120.22	68.31	105.24	132.06
11	64.26	89.56	124.47	74.38	105.24	129.53	77.92	113.85	145.72
12	72.35	100.69	132.57	83.49	114.35	136.62	87.03	124.98	154.8
13	76.35	105.24	138.64	88.04	121.44	142.69	91.58	128.01	160.9
14	78.43	107.27	140.16	90.57	123.97	145.22	94.11	131.05	163.94
15	79.44	108.28	140.66	91.08	124.98	146.23	95.38	131.56	165.46
V H_2	79.44	108.28	140.66	91.08	124.98	146.23	95.38	131.56	165.46
LSD at 5% = 9.55									

Table 2 presents the effect of a variable power supply for hydrogen yield and volumes of Bio-H_2 production from industrial wastewater in MECs. Power supply 0.8 V gave the highest hydrogen yield 71.94 % and the highest volume of Bio-H_2 165.46 cm^3 production at the anode chamber 500 ml, which revealed significant differences were found between this yield, volume and other yields and volumes. No significant differences were found between the hydrogen yield 67.76 % and 66.63 % also volume of Bio-H_2 146.23 cm^3 and 140.66 cm^3 production at the anode chamber 500 ml with power supply 0.6 V and 0.4 V respectively. Power supply 0.4 V the lowest hydrogen yield 48.02 % and the lowest volume of Bio-H_2 79.44 cm^3 production at the anode chamber 300 ml.

These results are consistent with those reported by Jia *et al.*, (2010) who also obtained the highest hydrogen yield and highest volume of Bio-H_2 from wastewater with increasing power supply to 0.8 V.

Table 2. Effect of power supply 0.4 V, 0.6 V and 0.8 V for Hydrogen yield (%) production from industrial wastewater in MECs.

Power supply (V)	MECs	Δ COD (mg / L)	VH_2 (cm^3)	$^nH_2$ (mol)	$^n th$ (mol)	YH_2 %
0.4 V	300 ml	105	79.44	3.15	6.56	48.02
	400 ml	119	108.28	4.29	7.43	54.12
	500 ml	134	140.66	5.58	8.37	66.63
0.6 V	300 ml	111	91.08	3.61	6.93	52.09
	400 ml	127	124.98	4.95	7.93	62.47
	500 ml	137	146.23	5.80	8.56	67.76
0.8 V	300 ml	113	95.38	3.78	7.06	53.58
	400 ml	130	131.56	5.22	8.12	64.24
	500 ml	146	165.46	6.56	9.12	71.94
LSD at 5%			9.55			2.65

Effect of power supply and *Enterobacter aerogenes* DSM 30053 for Hydrogen yield (%) and Bio-hydrogen production:

While in the case of used power supply and *Enterobacter aerogenes* DSM 30053 in MEC at the anode chamber 300, 400 and 500 ml for Hydrogen yield (%) and Bio-hydrogen produced. The hydrogen gas production started from second to third day. Power supply 0.8 V gave the lowest volume of Bio-H_2 183.17 cm^3 at the anode chamber 300 ml also gave the highest volume of Bio-H_2 348.1 cm^3 at the anode chamber 500 ml, which were significant differences found between this volume and other volumes of Bio-H_2 (cm^3) (Table 3).

The Bio-H_2 production in MECs at anode chamber (300 , 400 and 500 ml) by Enterobacter aerogenes DSM 30053 from industrial wastewater increased from third and fourth day till thirteenth day because of the biofilm around on the anode electrode which indicates the growth of *Enterobacter aerogenes* DSM 30053 which increasing industrial wastewater degradation rate. From fourteenth day the volume of Bio-H_2 started decreasing which indicates that industrial wastewater degradation rate is decreasing and the Bio-H_2 production stopped from fifteenth day.

These results are in agreement with Kiran and Gaur (2013) found that *Enterobacter aerogenes* able to degradation of organic matter in a variable substrates and electron transfer from anode to cathode electrodes in microbial fuel cell for electricity generation and bio-hydrogen production.

Table 3. Effect of power supply and *Enterobacter aerogenes* DSM 30053 for Bio-H_2 (cm^3) production at anode chamber 300, 400 and 500 ml from industrial wastewater .

Days	0.4 V for Bio-H_2 produced (cm^3) at anode chamber			0.6 V for Bio-H_2 produced (cm^3) at anode chamber			0.8 V for Bio-H_2 produced (cm^3) at anode chamber		
	300 ml	400 ml	500 ml	300 ml	400 ml	500 ml	300 ml	400 ml	500 ml
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	12.14	10.12	10.62	0.0	10.62	12.14	0.0	12.14	14.16
3	34.4	33.39	28.84	9.61	27.83	34.91	10.12	36.43	35.92
4	58.69	56.64	55.15	30.86	49.08	59.2	26.31	60.21	66.79
5	76.91	77.92	83.49	57.68	69.82	88.55	49.58	81.97	93.1
6	96.14	89.67	102.71	79.94	94.11	119.92	67.8	115.36	122.95
7	130.04	125.99	121.49	96.14	120.93	145.72	82.47	154.83	147.24
8	147.24	145.72	148.25	112.33	154.33	221.12	96.64	189.24	179.63
9	162.42	158.88	179.63	129.53	186.2	198.35	110.81	221.62	209.48
10	177.6	180.13	205.94	145.72	203.41	175.58	128.01	240.85	242.37
11	191.77	194.3	234.78	158.88	220.11	247.43	144.21	258.56	263.62
12	203.91	206.44	256.03	171.02	236.8	267.16	152.3	274.75	293.48
13	209.99	212.52	277.28	177.1	244.9	286.39	156.35	282.85	322.82
14	213.53	215.55	280.32	181.65	248.95	289.93	158.37	287.91	336.99
15	215.55	217.07	281.84	183.17	250.97	292.8	159.39	288.92	348.1
V H_2	215.55	217.07	281.84	183.17	250.97	292.8	159.39	288.92	348.1
LSD at 5% = 20.95									

The highest hydrogen yield 92.86 % and the highest volume of Bio-H_2 348.1 cm^3 production by *Enterobacter aerogenes* DSM 30053 in MECs at the anode chamber 500 ml with power supply 0.8 V obtained from industrial wastewater, which revealed significant differences were found between other yields and volumes. No significant differences were found between volume of Bio-H_2 292.8 cm^3 , 281.84 cm^3 at the anode chamber 500 ml with power supply 0.6 V and 0.4 V respectively and volume of Bio-H_2 288.84 cm^3 at the anode chamber 400 ml with power supply 0.8 V. The lowest hydrogen yield 70.02 % and the lowest volume of Bio-H_2 159.39 cm^3 production at the anode chamber 300 ml with power supply 0.8 V, which revealed significant negative relationship between the hydrogen yield, volumes of Bio-H_2 and all power supply (Table 4).

These results are consistent with those reported by Jia *et al*., (2010) who also obtained the highest hydrogen yield and highest volume of Bio-H_2 from wastewater with increasing power supply to 0.8 V.

Bacteria was more effecting from power supply when Bio-hydrogen production in MECs because the highest hydrogen yield 92.86 % and the highest volume of Bio-H_2 348.1 cm^3 production by *Enterobacter aerogenes* DSM 30053 with power supply 0.8 V at the anode chamber 500 ml. While the highest hydrogen yield 71.94 % and the highest volume of Bio-H_2 production 165.46 cm^3 without addition of bacteria at the same condition. Table (5) present the hydrogen yield and volume of Bio-H_2 production from industrial wastewater in MECs by bacteria and without addition of bacteria.

Table 4. Effect of power supply and *Enterobacter aerogenes* DSM 30053 for Hydrogen yield (%) production from industrial wastewater in MECs .

Power supply (V)	MECs	Δ COD (mg / L)	VH_2 (cm^3)	$^nH_2$ (mol)	^{n}th (mol)	YH_2 %
0.4 V	300 ml	172	215.55	8.55	10.75	79.56
	400 ml	173	217.07	8.61	10.81	79.66
	500 ml	205	281.84	11.18	12.81	87.26
0.6 V	300 ml	156	183.17	7.26	9.75	74.54
	400 ml	190	250.97	9.95	11.87	83.86
	500 ml	210	292.8	11.61	13.12	88.52
0.8 V	300 ml	144	159.39	6.32	9	70.02
	400 ml	190	288.92	11.46	13.06	87.77
	500 ml	238	348.1	13.81	14.87	92.86
LSD at 5%			20.96			2.53

The significant positive relationship obtained in the present study between the hydrogen yield, volume of Bio-H_2 production and industrial wastewater revealed that is good substrate for Bio-H_2 production in MECs.

Table 5. Comparison of Hydrogen yield (%) and volume of Bio-H_2 (cm^3) produced with and without *Enterobacter aerogenes* DSM 30053 from industrial wastewater:

Power supply (V)	MECs	Without bacteria		*Enterobacter aerogenes* DSM 30053	
		VH_2 (cm^3)	YH_2 %	VH_2 (cm^3)	YH_2 %
0.4 V	300 ml	79.44	48.02	215.55	79.56
	400 ml	108.28	54.12	217.07	79.66
	500 ml	140.66	66.63	281.84	87.26
0.6 V	300 ml	91.08	52.09	183.17	74.54
	400 ml	124.98	62.47	250.97	83.86
	500 ml	146.23	67.76	292.8	88.52
0.8 V	300 ml	95.38	53.58	159.39	70.02
	400 ml	131.56	64.24	288.92	87.77
	500 ml	165.46	71.94	348.1	92.86
LSD at 5%		9.55	2.65	20.96	2.53

These results are confirm with Lu *et al*., (2012) they used *Enterobacter aerogenes* for bio-hydrogen production from glucose as substrate in microbial electrolysis cells at low temperature. Also, Afify, *et al*., (2017) reported that the bio-hydrogen production in MECs effect when addition enteric bacteria (*E.coli* NRRL B – 3008).

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تأثير الجهد الكهربى والبكتيريا على إنتاج الهيدروجين الحيوى بإستخدام خلايا التحليل الكهربى الميكروبية

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الجهد الكهربى الخارجى والبكتيريا من أهم العوامل تأثيراً على إنتاج الهيدروجين الحيوى . ففى هذه الدراسة تم إستخدام 0.4 و 0.6 و 0.8 فولت كجهد كهربى خارجى لإنتاج الهيدروجين الحيوى فى خلايا التحليل الكهربى الميكروبية بواسطة إضافة وكذلك عدم إضافة بكتيريا الـ *Enterobacter aerogenes* DSM 30053 .وذلك بإستخدام مياة الصرف الصناعى كمادة لإنتاج الهيدروجين الحيوى ، فوجد ان أعلى إنتاج من الهيدروجبن الحيوى الناتج من استخدام مياه الصرف الصناعى348.1 سم ^{3} بنسبة 92.86 % عند جهد كهربى خارجى 0.8 و غرفة أنود 500 ملليتر وذلك عند إضافة البكتيريا . بينما أعلى إنتاج من الهيدروجبن الحيوى الناتج عند عدم إضافة البكتيريا هو 165.46 سم ^{3} بنسبة 71.94 % عند جهد كهربى خارجى 0.8 و غرفة أنود 500 ملليتر.