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Bio-hydrogen Production by *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 Using Microbial Electrolysis Cells

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ABSTRACT

Industrial wastewater was used as the substrate for bio-hydrogen (Bio-H_2) production in Microbial Electrolysis Cells (MECs) by *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853. Three volumes of anode chamber in MECs 300ml, 400 ml and 500 ml were applied. Salt bridge was used for the exchange of protons from anode to cathode chamber. External voltage of 0.4 V, 0.6 V and 0.8 V was used applied to MECs using a regulated power supply. The highest volume of Bio-H_2 358.24 cm^3 and 343.57cm^3 at the anode chamber 500 ml with power supply 0.8 V by *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 respectively .

Keywords: Industrial wastewater, Bio-hydrogen, Microbial electrolysis cells, *Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853

ABBREVIATIONS : Carbon brush (No. 34 D) = Degree of conductivity ; DC= Direct current; Hz = Hertz ; mA = ml Ampere; mS = ml Siemens ; NTU = Nephelometric turbidity meter; Stainless steel (304)= degree of corrosion resistance ; V = Voltage

INTRODUCTION

In Egypt, dependence on fossil fuels as a primary energy source up to 90% leads to global climate change and problems of human health. Moreover, the recent rise petrol and natural gas prices may drive the Egypt economy toward find an alternative clean energy. Hydrogen has been identified as an alternative clean energy. Hydrogen is a green fuel that is almost free of CO_2 and other pollutant emissions due to its oxidation product is only H_2O vapor. Hydrogen is high calorific value fuel (120–142 MJ/kg). Hydrogen can be derived from a wide variety of renewable feedstock and domestic waste materials. Kumar and Lin (2013), Hernandez and Buitron (2013) and Lai *et al.,* (2014).

Bio-hydrogen is the hydrogen produced by biological process from biomass and waste materials such as bio-photolysis by algae , photo fermentation by green algae and cyanobacteria , dark fermentation by bacteria and MECs Kotay and Das (2008).

MEC as bio-electrochemical reactor is novel biological process for Bio-H_2 production from wastewater as substrate. Operation of MEC relies on the presence of bacteria for degraded the substrate at anode chamber into CO_2 , protons, electrons and residual organic matter. Electrons transfer by bacteria to anode chamber and released to cathode chamber through circuit and protons pass to cathode across salt bridge. When protons meet with electrons at cathode, hydrogen can be produced with a regulated power supply (RPS) was used for applying the external voltage to MEC Liu *et al.,* (2005), Lalaurette *et al.,* (2009) and Montpart *et al.,* (2015)

This study aim to Bio-H_2 production from industrial wastewater as substrate using MECs as bioreactor for Bio-H_2 production. By *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 for degraded organic matter in substrate at anode chamber in MECs, power supply acts as external voltage to MECs on volume of Bio-H_2 gas.

MATERIALS AND METHODS

The present experiments were conducted in Microbiology Dept., Desert Res. Center, Matariya, Cairo, Egypt.

Bacteria used :

Escherichia coli NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 were obtained from microbiological resource center (Cairo MIRCEN), Faculty of Agriculture , Ain Shams University, Cairo, Egypt.

Bacteria preparation :

Escherichia coli NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 were grown for 24 – 48 hr. at 30 – 37 ^oC in nutrient broth medium. Two conical flasks were prepared by adding 5.2 gm of nutrient broth powder to 400 ml of distilled water in a 500ml conical flask. These conical flasks were autoclaved for 15 min. at 121 ^oC under pressure. *Escherichia coli* NRRL B-3008 was inoculated to the first conical flask and *Pseudomonas aeruginosa* ATCC 27853 was inoculated to the second flask in LAF (Laminar Air Flow). These conical flasks were incubated in the orbital shaker for 72 hr. The clear solution turned turbid which indicates bacteria growth. The cultures were stored in a refrigerator at 12 oC until it was used. All this process done under sterile conditions. Nivedhan *et al.,* (2014)and Rakesh *et al.,* (2014).

Substrate :

Industrial wastewater were obtained from Pickles Factory – small industrial area, EL-Obour city – EL-Qaliubiya governorate, Eygpt. For microbes to survive in the cell the pH should be adjusted 7 by using 1M NaOH and 0.2M sodium phosphate buffer solution. The characteristics of industrial wastewater used in the experiments are depicted in Table 1 .

Table 1 . The characteristics of industrial wastewater.

Test	Industrial wastewater
pH	4.4
Color	Tangerine
Turbidity (NTU)	723
Electrical conductivity (ms)	74.9
Organic matter (%)	19.78
Total dissolved solids (mg / L)	4793
Chemical oxygen demand (mg / L)	276
Biochemical oxygen demand (mg / L)	854

MEC construction :

Two-chamber MEC is commonly used design consisting of two chambers with anode and cathode chambers separated by salt bridge as cation / anion exchange

membrane, which allows protons to transfer across to cathode chamber. The chambers can be bottle type (MEC H-type). Each chamber has a volume of 300 ml , 400 ml and 500 ml. Anode chamber contains carbon brush (No.34 D) plate as anode electrode. Cathode chamber which contains stainless steel (304) as cathode electrode. Anode electrode and cathode electrode were connected to power supply. The construction of two chambers MEC is shown in Fig.1. Ujwal *et al.,* (2015) and Kadier *et al.,* (2016).

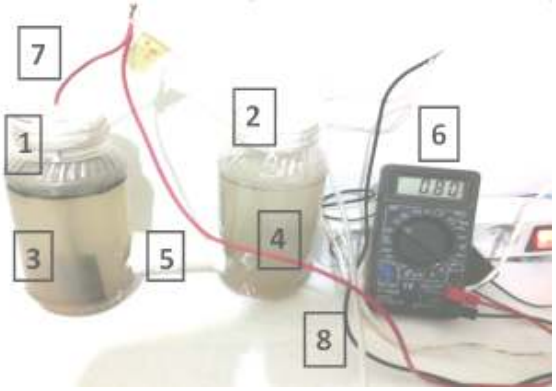

Fig. 1. MEC construction ; (1) Anode chamber filled with substrate and bacteria ; (2) Cathode chamber filled with distilled water ; (3) Anode electrode " carbon brush" ; (4) Cathode electrode " stainless steel " ; (5) Salt bridge ; (6) Power supply = 0.8 V ; (7)Copper wire was connected between electrodes ; (8) Tube hydrogen exit.

Salt bridge as membrane :

Salt bridge consist of agar (20gm / L) and 1M of potassium chloride (KCl 174.5 gm / L) Potassium chloride (KCl) as strong salt was tested for efficacy to transport hydrogen ions (protons) in the salt bridge. The purpose of an agar salt bridge is to provide an electrical connection to both solutions in two chambers and salt bridge allows hydrogen ions to transfer from anode to cathode chamber to maintain a balance in charge between chambers Anand (2015)

MEC start up and operation :

The anode chamber was filled with as substrates (300 ml, 400 ml and 500 ml of industrial wastewater) 30 ml, 40 ml and 50 ml (10 % v/v) of bacterial culture was added to the anode chamber which contains carbon brush plate as the anode (positive) electrode. 300 ml , 400 ml and 500 ml of distilled water was added to the cathode chamber which contains stainless steel plate as the cathode (negative) electrode. Copper wire connected between electrodes, carbon brush electrode was connected to the positive terminal of the regulated power supply (0.4 V, 0.6 V and 0.8 V / 500 mA / DC / 50 Hz) while the stainless steel electrode was connected to the negative terminal of the regulated power supply thus the circuit completed.

Volume of Biohydrogen (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)

Volume of Bio-H_2 (cm^3) was calculated as:

Volume of Bio-H_2 (cm^3) = length of burette reading (cm) × inner surface area of burette tube (cm^2)

= length of burette reading (cm) × π r^2 (cm^2)

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

Statistical analysis :

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

RESULTS AND DISCUSSION

MECs for Bio-hydrogen Production :

In MECs industrial wastewater was used as substrate in anode chamber to produce bio-hydrogen. Industrial wastewater degraded by bacteria into electron and protons. Bacterial strains transfer the electrons to the anode chamber which are released to the cathode chamber through the circuit and the protons pass to the cathode across salt bridge. When protons combine with electrons at the cathode, hydrogen can be generated at the cathode chamber according to :

$$2H^+ + 2\,e^- \rightarrow H_2$$

MECs operation relies on the presence of bacteria in anode chamber under anaerobic conditions in cathode chamber. *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 using in MECs for degradation organic matter in industrial wastewater with 0.4 V, 0.6 V and 0.8 V/ 500 mA / DC / 50 HZ as a regulated power supply was used as the external voltage to the MEC .

Bio-hydrogen Production In MECs By *Escherichia coli* NRRL B-3008 :

Using industrial wastewater as the substrate in MECs, *Escherichia coli* NRRL B-3008 which degraded organic matter in industrial wastewater, organic matter consists mainly of carbohydrates, fats and proteins . *Escherichia coli* NRRL B-3008 utilized carbohydrates as carbon source, then released electron and proton, electron was transferred to anode then to cathode by external circuit (power supply) , meantime, proton moved to cathode chamber through salt bridge then at the cathode it combined with electron and generated hydrogen. *Escherichia coli* NRRL B-3008 can self-mediate the extracellular electron transfer to electrode in MECs . *Escherichia coli* NRRL B-3008 has been used as bacterial strain in many MECs, which has advantages of easy access, easy cultivation, low cost, safety, and metabolizing a variety of substrates Liu *et al.,* (2012)

30 ml, 40 ml and 50 ml of *E. coli* NRRL B-3008 bacterial culture was added to 300 ml, 400ml and 500 ml of industrial wastewater at the anode chamber respectively. The hydrogen gas production in MEC (anode chamber 300 ml) started from second day with power supply 0.4 V and 0.8 V and third day with power supply 0.6 V onwards till fifteenth day. Significant differences between highest volume of Bio-H_2 222.26 cm^3 with power supply 0.8 V and other volumes of Bio-H_2 190.76 cm^3 and 206.95 cm^3 with power supply 0.4 V and 0.6 V respectively (Table 2).

The present results are in agreement with those reported by Jia *et al.,* (2010) who investigated the bio-hydrogen can be produced using MECs with power supply over 0.4 V and bio-hydrogen gradually increased with increasing power supply.

Table 2. Volume of Bio-H_2 (cm^3) collected in MECs (Anode chamber 300 ml) by *E. coli* NRRL B-3008 from industrial wastewater with a variable power supply (0.4 V, 0.6 V and 0.8 V).

Power supply	0.4 V		0.6 V		0.8 V	
Days	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)
1	0.0	0.0	0.0	0.0	0.0	0.0
2	2.2	11.13	0.0	0.0	2.7	13.66
3	5.4	27.32	2.4	12.14	6.9	34.91
4	9.6	48.57	6.2	31.37	11.6	58.69
5	12.1	61.22	9.7	49.08	14.8	74.88
6	16.5	83.49	13.4	67.8	20.4	103.22
7	20.7	104.74	17.2	87.03	24.5	123.97
8	23.6	119.41	22.4	113.34	28.2	142.69
9	28.3	143.19	30.5	154.33	33.8	171.02
10	31.9	161.41	34.2	173.05	36.9	186.71
11	35.4	179.12	37.9	191.77	41.1	207.96
12	36.6	185.19	39.5	199.87	41.5	215.05
13	37.2	188.23	40.3	203.91	43.2	218.59
14	37.5	189.75	40.7	205.94	43.7	221.12
15	37.7	190.76	40.9	206.95	44	222.64
V H_2		190.76		206.95		222.64
LSD at 5%				4.99		

In MEC at the anode chamber 400 ml hydrogen gas production started from second day with power supply 0.8 V and third day with power supply 0.4 V and 0.6 V . The highest volume of Bio-H_2 291.96 cm^3 with power supply 0.8 V was significantly greater than other volumes. No significant differences was found between the lowest volume of Bio-H_2 279.81cm^3 with power supply 0.4 V and volume of Bio-H_2 284.37 cm^3 with power supply 0.6 V (Table 3).

These results are consistent with those reported by Liu *et al.,* (2012) who stated that *E. coli* was able to degradation organic matter in industrial wastewater and electron transfer to electrodes in MEC for bio-hydrogen production.

Table 3 . Volume of Bio-H_2 (cm^3) collected in MECs (Anode chamber 400 ml) by *E. coli* NRRL B-3008 from industrial wastewater with a variable power supply (0.4 V, 0.6 V and 0.8V).

Power supply	0.4 V		0.6 V		0.8 V	
Days	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	2.1	10.62
3	1.9	9.61	3.4	17.2	8.6	43.51
4	5.4	27.32	8.8	44.52	14.5	73.37
5	12.9	65.27	15.3	77.41	20.2	102.21
6	19.6	99.17	22.8	115.36	26.3	133.07
7	25.8	130.54	30.1	152.3	31.8	160.9
8	32.1	162.42	35.5	179.63	38.2	193.29
9	37.5	189.75	39.9	201.89	43.3	219.09
10	45.7	231.24	43.6	220.61	47.8	241.86
11	48.7	246.42	48.9	247.43	53.7	271.72
12	54.1	273.74	54.9	277.79	56.2	284.37
13	54.7	276.78	55.7	281.84	57.1	288.92
14	55.1	278.8	56.2	284.37	57.5	290.95
15	55.3	279.81	56.2	284.37	57.7	291.96
V H_2		279.81		284.37		291.96
LSD at 5%				4.65		

While in the case of used MEC at the anode chamber 500 ml the hydrogen gas production started from second day with all power supply but the highest volume of Bio-H_2 358.24 cm^3 with power supply 0.8 V, which were significant differences found between this volume and other volumes of Bio-H_2 321.8 cm^3 and 336.99 cm^3 with power supply 0.4 V and 0.6 V respectively (Table 4).

The Bio-H_2 production in MECs at the anode chamber 300 ml , 400 ml and 500 ml increased from second and third day till thirteenth day because of the biofilm around the anode electrode which indicates the growth of *Escherichia coli* NRRL B-3008 which increasing the substrate degradation rate. From the fourteenth day the volume of Bio-H_2 started decreasing which indicates that the substrate degradation rate is decreasing and the Bio-H_2 production stopped from fifteenth day.

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 wastewater from sugar industry for bio-hydrogen production in MECs.

Table 4 . Volume of Bio-H_2 (cm^3) collected in MECs (Anode chamber 500 ml) by *E. coli* NRRL B-3008 from industrial wastewater with a variable power supply (0.4 V, 0.6 V and 0.8V).

Power supply	0.4 V		0.6 V		0.8 V	
Days	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)
1	0.0	0.0	0.0	0.0	0.0	0.0
2	2.3	11.63	2.1	10.62	2.9	14.67
3	4.6	23.27	5.8	29.34	7.5	37.95
4	10.8	54.64	12.3	62.23	14.2	71.85
5	17.7	89.56	21.4	108.28	20.7	104.74
6	24.3	122.95	27.5	139.15	29.5	149.27
7	31.9	161.41	35.6	180.13	38.7	195.82
8	37.6	190.25	41.6	210.49	46.6	235.79
9	44.9	227.19	47.3	239.33	54.9	277.79
10	50.2	254.01	53.1	268.68	60.4	305.62
11	55.7	281.84	57.9	292.97	64.8	327.88
12	60.9	308.15	61.7	312.2	67.6	342.05
13	62.5	316.25	64.5	326.37	69.5	351.67
14	63.4	320.8	66.3	335.47	70.7	357.74
15	63.4	321.8	66.6	336.99	70.8	358.24
V H_2		321.8		336.99		358.24
LSD at 5%				2.13		

Bio-hydrogen Production In MECs By *Pseudomonas aeruginosa* ATCC 27853:

Pseudomonas aeruginosa is widely distributed in nature, the most common bacteria in the waste water. When using *Pseudomonas aeruginosa* ATCC 27853 to degrade organic matter, the MECs is more effective than the conventional anaerobic culture method. Study clearly indicates that *Pseudomonas aeruginosa* ATCC 27853 can metabolize organic matter as a source of carbon and nitrogen.

Also when using *Pseudomonas aeruginosa* ATCC 27853 for Bio-H_2 production in MECs were added 30 ml, 40 ml and 50 ml of *Pseudomonas aeruginosa* ATCC 27853 bacterial culture to 300 ml, 400ml and 500 ml of industrial wastewater at the anode chamber. Table 5 presents the volume of hydrogen gas production in MEC at the anode chamber 300 ml. Hydrogen gas started from second day with power supply 0.4 V and third day with power supply 0.6 V and 0.8 V. Analysis of volumes of Bio-H_2 with a variable power supply revealed significant differences between used a variable power supply. 0.4 V was superior to 0.6 V and 0.8 V. Highest volume of Bio-H_2 230.73 cm^3 with power supply 0.4 V, while lowest volume of Bio-H_2 219.6 cm^3 with power supply 0.8 V.

These results are in agreement with Cao *et al.,* (2014) found that *Pseudomonas aeruginosa* able to degradation of organic matter in a variable substrates and electron transfer to electrodes in microbial fuel cell for electricity generation and bio-hydrogen production.

Table 5. Volume of Bio-H_2 (cm^3) collected in MECs (Anode chamber 300 ml) by *Pseudomonas aeruginosa* ATCC 27853 from industrial wastewater with a variable power supply (0.4 V, 0.6 V and 0.8 V).

Power supply	0.4 V		0.6 V		0.8 V	
Days	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)
1	0.0	0.0	0.0	0.0	0.0	0.0
2	1.8	9.1	0.0	0.0	0.0	0.0
3	4.5	22.77	2.7	13.66	2.1	10.62
4	8.4	42.5	5.2	26.31	4.6	23.27
5	13.6	68.81	9.9	50.09	7.8	39.46
6	18.5	93.61	14.8	74.88	12.1	61.22
7	23.1	116.88	19.4	98.16	16.6	83.99
8	27.6	139.65	23.7	119.92	20.8	105.24
9	33.4	169	27.2	137.63	26.3	133.07
10	37.5	189.75	32.5	164.45	31.5	159.39
11	41.1	207.96	36.7	185.7	35.9	181.65
12	43.5	220.11	40.3	203.91	39.1	197.84
13	44.7	226.18	43.7	211.12	42.8	216.56
14	45.3	229.21	44.2	223.65	43.2	218.59
15	45.6	230.73	44.3	224.15	43.4	219.6
V H_2		230.73		224.15		219.6
LSD at 5%				2.38		

While the anode chamber 400 ml the hydrogen gas production started from second day with power supply 0.8 V and third day with power supply 0.4 V and 0.6 V. Significant differences were found between highest volume of Bio-H2 263.12 cm3 with power supply 0.8 V and other volumes of Bio-H2 250.79 cm3 is the lowest volume with power supply 0.4 V (Table 6).

These results are confirm with Ujwal et al., (2015) they found that the highest volume of bio-hydrogen obtained from industrial wastewater by *Pseudomonas aeruginosa* was obtained with power supply 0.8 V using MEC.

Table 6 . Volume of Bio-H_2 (cm^3) collected in MECs (Anode chamber 400 ml) by *Pseudomonas aeruginosa* ATCC 27853 from industrial wastewater with a variable power supply (0.4 V, 0.6 V and 0.8V).

Power supply	0.4 V		0.6 V		0.8 V	
Days	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	2.7	13.66
3	2.5	12.65	3.4	17.2	8.1	40.98
4	5.9	29.85	6.9	34.91	8.1	66.79
5	10.4	52.62	11.5	58.19	19.7	99.68
6	15.8	79.94	18.9	95.63	23.8	120.42
7	20.9	105.75	22.4	113.34	28.5	144.21
8	26.1	132.06	28.6	144.71	34.9	176.59
9	30.8	155.84	33.3	168.49	37.2	188.23
10	35.4	179.12	37.6	190.25	41.8	211.5
11	41.1	207.96	42.9	217.07	45.6	230.13
12	44.6	225.67	46.1	233.26	49.1	248.44
13	48.7	246.42	48.5	245.41	50.6	256.03
14	49.2	248.95	50.3	254.51	51.7	261.6
15	49.6	250.79	50.4	255.02	52	263.12
V H_2		250.79		255.02		263.12
LSD at 5%				2.64		

But when using anode chamber 500ml the hydrogen gas production started from second day with power supply 0.4V, 0.6 V and 0.8 V. The highest volume of Bio-H_2 343.57 cm^3 with power supply 0.8 V referring to significant differences were found between the highest volume of Bio-H_2 and volumes 292.46 cm^3 and 329.4 cm^3 with power supply 0.4 V and 0.6 V respectively (Table 7).

In MECs at the anode chamber 300 ml , 400 ml and 500 ml the Bio-H_2 production increased from second and third day till thirteenth day because of the biofilm around the anode electrode which indicates the growth of *Pseudomonas aeruginosa* ATCC 27853 which increasing the substrate degradation rate. The Bio-H_2 production stopped from fourteenth and fifteenth day.

These results are agreed with the results obtained by Nivedhan *et al.,* (2014), who stated that *Pseudomonas aeruginosa* able to produced the highest volume of bio-hydrogen during 15 days from glycerol as substrate using MEC with power supply 0.8 V.

Table 7. Volume of Bio-H_2 (cm^3) collected in MECs (Anode chamber 500 ml) by *Pseudomonas aeruginosa* ATCC 27853 from industrial wastewater with a variable power supply (0.4 V, 0.6 V and 0.8V).

Power supply	0.4 V		0.6 V		0.8 V	
Days	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)	Burette reading (cm)	Volume of Bio-H_2 collected (cm^3)
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.8	4.04	1.9	9.61	2	10.12
3	3.7	18.72	4.4	22.26	4.6	23.27
4	9.1	46.05	8.9	45.03	11.3	57.17
5	15.3	77.41	16.2	81.97	20.1	101.7
6	23.7	119.92	24.5	123.97	29.8	130.45
7	29.1	147.24	33.4	169	35.4	179.12
8	35.5	179.63	39.5	199.87	44.5	225.17
9	44.8	226.68	45.7	231.24	49.6	250.97
10	48.3	244.39	51.6	261.09	53.1	268.68
11	51.4	260.08	56.4	285.38	60.7	307.14
12	54.6	276.27	61.4	310.68	64.2	324.85
13	56.9	287.91	64.6	326.87	67.1	339.52
14	57.5	290.95	46.8	327.88	67.8	343.06
15	57.8	292.46	65.1	329.4	67.9	343.57
V H_2		292.46		329.4		343.57
LSD at 5%				4.21		

Finally it is clear that Bio-H_2 production by *Escherichia coli* NRRL B-3008 is better than *Pseudomonas aeruginosa* ATCC 27853 from industrial waste water using microbial electrolysis cells (MECs) with a variable power supply and a variable volumes of anode chamber.

Analysis of volume of Bio-H_2 production by *Escherichia coli* NRRL B-3008 revealed significant differences were found between the highest volume of Bio-H_2 358.24 cm^3 at the anode chamber 500ml with power supply 0.8 V and other volumes of Bio-H_2 at the anode chamber 300ml,400 ml and 500 ml with a variable power supply. No significant differences were found between all volumes of Bio-H_2 at the anode chamber 300 ml and 400 ml. with the same volumes of Bio-H_2 production by *Pseudomonas aeruginosa* ATCC 27853. The highest volume of Bio-H_2 343.57cm^3 production by *Pseudomonas aeruginosa* ATCC 27853 with power supply 0.8 V at anode chamber 500 ml referring to significant differences were found between this volume and other volumes of Bio-H_2 at the anode chamber 300ml, 400 ml and 500 ml. (Table 8).

Table 8. Comparison of volume of Bio-H_2 (cm^3) in MECs with *Escherichia coli* NRRL B-3008 and *Pseudomonas aeruginosa* ATCC 27853 from industrial waste water.

MECs	Power supply (V)	Volume of Bio-H_2 (cm^3) By *Escherichia coli* NRRL B-3008	Volume of Bio-H_2 (cm^3) By *Pseudomonas aeruginosa* ATCC 27853
Anode chamber 300ml	0.4 V	190.76	230.73
	0.6 V	206.95	224.15
	0.8 V	222.64	219.6
Anode chamber 400ml	0.4 V	279.81	250.97
	0.6 V	284.37	255.02
	0.8 V	291.96	263.12
Anode chamber 500ml	0.4 V	321.8	292.46
	0.6 V	336.99	329.4
	0.8 V	358.24	343.57
LSD at 5%		19.54	10.68

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إنتاج الهيدروجين الحيوى ببكتيريا الـ *Escherichia coli* NRRL B-3008 و بكتيريا الـ *Pseudomonas aeruginosa* ATCC 27853 باستخدام خلايا التحليل الكهربى الميكروبية (MECs)

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الهدف من هذه الدراسة استخدام مياة الصرف الصناعى لانتاج الهيدروجين الحيوى بواسطة نوعين من البكتيريا وهما *Escherichia coli* NRRL B-3008 وبكتيريا الـ *Pseudomonas aeruginosa* ATCC 27853 باستخدام خلايا التحليل الكهربى الميكروبية (MECs) ، حيث تم استخدام ثلاثة احجام مختلفة من غرفة الأنود لخلية التحليل الكهربى الميكروبية وهى 300 مل ، و400 مل ، 500 ملليتر مع استخدام القنطرة الملحية لتبادل ايونات الهيدروجين من غرفة الأنود إلى غرفة الكاثود فى خلية التحليل فى وجود الجهد الكهربى الخارجى 0.4 فولت ، و 0.6 فولت ، 0.8 فولت كقوة داعمة لنقل الإلكترون ، فوجد ان أعلى إنتاج من الهيدروجين الحيوى كان باستخدام بكتيريا الـ *Escherichia coli* NRRL B-3008 و بكتيريا الـ *Pseudomonas aeruginosa* ATCC 27853 وهو 358.24 سم ^{3} و343.57 سم ^{3} على التوالى عند غرفة أنود 500 ملليتر وجهد كهربى خارجى 0.8فولت .