J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 8 (6): 167 – 171, 2017

Using *Enterobacter aerogenes* DSM 30053 for Bio-hydrogen Production by Microbial Electrolysis Cells from Domestic Wastewater

Aida H. Afify[1]; A. M. Abd EL Gwad[2] and N. K. Abd EL Rahman[2]
[1] Microbiology Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt.
[2] Soil Fertility and Microbiology Dept., Desert Res. Center, Matariya, Cairo, Egypt.

ABSTRACT

Microbial electrolysis cells (MECs) were used for production of bio-hydrogen (Bio-H_2)by *Enterobacter aerogenes* DSM 30053 from domestic wastewater at three volumes of anode chamber in MECs 300ml, 400 ml and 500 ml were applied. Power supply of 0.4 V, 0.6 V and 0.8 V was used applied to MECs using a regulated external voltage. The highest volume of Bio-H_2 112.83 cm^3 production was obtained from domestic wastewater without addition of bacteria at the anode chamber 500 ml with power supply 0.8 V. While the highest volume of Bio-H_2 316 cm^3 production was obtained by *Enterobacter aerogenes* DSM 30053 at the anode chamber 500 ml with power supply 0.4 V from domestic wastewater.

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

INTRODUCTION

Domestic wastewater was used as substrate in microbial electrolysis cells at the anode chamber to produce bio-hydrogen in cathode chamber. Bacteria degraded organic matter in domestic wastewater into electrons and protons. The electrons were transferred by bacterial strains to the anode chamber which released to the cathode chamber through the circuit, protons pass to the cathode across membrane or salt bridge and combine with electrons in cathode chamber for production hydrogen. Montpart *et al.,* (2015)

Enterobacter aerogenes is gram-negative and facultative anaerobic. It is live in various wastewater, pH 6-7 is optimum value of pH for bio-hydrogen production, which have the capability to transfer electrons from inside the cell to the extracellular acceptors through c-type cytochromes and microbial nanowires (flagella and pili) present on their outer membrane. *Enterobacter aerogenes* was form biofilm on the anode electrode in MECs and act as electron acceptors and transfer electrons to the anode from bio-catalytic reactions in bio-electrochemical reactors. Kiran and Gaur (2013)

In this study domestic wastewater was used as substrate for Bio-H_2 production with and without bacteria. *Enterobacter aerogenes* DSM 30053 was used in MECs for degraded organic matter in domestic wastewater at anode chamber. Salt bridge was used as membrane in MEC. The effect of volume of anode chamber and power supply as the external voltage to the MECs on volume of bio-hydrogen gas.

MATERIALS AND METHODS

Bacteria used and preparation:

Nutrient broth medium (13gm / liter of distilled water) was used for preparation of bacterial culture, *Enterobacter aerogenes* DSM 30053 was obtained from microbiological resource center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Substrate preparation:

Domestic wastewater were obtained from EL-Berka wastewater treatment plant, EL-Khanka city, EL-Qaliubiya Governorate, Egypt. Drops of 1M HCl acid was added to the substrate for adjusted pH to 7 and 0.2M sodium phosphate buffer solution .All analyzes of domestic wastewater were carried out in the Central Lab at the Desert Research Center(Abd El Rahman, 2017) . The characteristics of domestic wastewater used in the experiments are depicted in Table 1.

Table 1. The characteristics of domestic wastewater.

Test	Domestic wastewater
pH	7.4
Color	Gray
Turbidity (NTU)	75.8
Electrical conductivity (ms)	10.42
Organic matter (%)	16.35
Total dissolved solids (mg / L)	3660
Chemical oxygen demand (mg / L)	243
Biochemical oxygen demand (mg / L)	552

MEC design and operation:

Design of MEC 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 ml, 400 ml 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 domestic wastewater (300 ml, 400 ml and 500 ml) and 30 ml, 40 ml and 50 ml (10 % v/v) of bacterial culture. Cathode chamber was filed by 300 ml, 400 ml 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).

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)

$$\text{Volume of Bio-H2 (cm3)} = \text{length of burette reading (cm)} \times \pi\ r2\ (cm2)$$

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 means values.

RESULTS AND DISCUSSION

Bio-hydrogen Production from domestic wastewater without bacteria:

Domestic wastewater were filled in anode chamber (300 ml, 400 ml and 500 ml) and distilled water in cathode chamber (300 ml, 400 ml and 500 ml) respectively. At the anode chamber 300 ml hydrogen gas was produced in cathode chamber started from sixth day with power supply 0.6 V and 0.8 V and seventh day with power supply 0.4 V. onwards till fifteenth day. Significant differences were found between the highest volume of Bio-H2 54.64 cm3 with power supply 0.8 V and lowest volume of Bio-H2 41.49 cm3 with power supply 0.4 V. But no significant differences were found between the volume of Bio-H2 54.64 cm3 and volume of Bio-H2 52.11 cm3 with power supply 0.6 V (Table2).

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

Table 2. The anode chamber 300 ml for Bio-H_2 (cm^3) production with power supply (0.4 V, 0.6 V and 0.8V) in MECs from domestic wastewater without bacterial addition.

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
2	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0
4	0	0.0	0	0.0	0	0.0
5	0	0.0	0	0.0	0	0.0
6	0	0.0	1.2	6.07	1.5	7.59
7	1.5	7.59	2.4	12.14	2.6	13.15
8	2.2	11.13	3.8	19.22	3.9	19.73
9	3.6	18.21	5.2	26.31	5.4	27.32
10	5.2	26.31	6.8	34.40	7	35.42
11	6.8	34.40	8.2	41.49	8.6	43.51
12	7.5	37.95	9.1	46.04	9.8	49.58
13	7.9	39.97	9.7	49.08	10.4	52.62
14	8.1	40.98	10.1	51.1	10.7	54.14
15	8.2	41.49	10.3	52.11	10.8	54.64
V H_2	41.49		52.11		54.64	
LSD at 5%			3.57			

The hydrogen gas was started production from fourth day with power supply 0.6 V and 0.8 V at the anode chamber 400 ml and fifth day with power supply 0.4 V. No significant differences were found between the highest volume of Bio-H_2 90.57 cm^3 with power supply 0.8 V and volume of Bio-H_2 87.03 cm^3 with power supply 0.6 V. The lowest volume of Bio-H_2 74.88 cm^3 with power supply 0.4 V was significantly greater than other volumes (Table3).

The present results are in agreement with those reported by Ivanov *et al.,* (2013) who developed simple methods for comparing the performance of different types of wastewaters and investigated the highest volumes of Bio-H_2 production from domestic wastewater in MECs operated under fed-batch operation mode.

Table 3. The anode chamber 400 ml for Bio-H_2 (cm^3) production with power supply (0.4 V, 0.6 V and 0.8V) in MECs from domestic wastewater without bacterial addition.

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
2	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0
4	0	0.0	1.9	9.61	2.2	11.13
5	1.7	8.6	3.5	17.71	4.1	20.74
6	3.4	17.2	5.4	27.32	5.9	29.85
7	5.2	26.31	7.3	36.93	7.5	37.95
8	6.8	34.4	8.9	45.03	9.2	46.55
9	8.4	42.5	11.1	56.16	10.9	55.15
10	10.2	51.61	12.5	63.25	12.7	64.26
11	11.8	59.7	14.3	72.35	14.4	72.86
12	13.4	67.8	15.9	80.45	15.8	79.94
13	14.2	71.85	16.7	84.5	17	86.02
14	14.6	73.87	17.1	86.52	17.6	89.05
15	14.8	74.88	17.2	87.03	17.9	90.57
V H_2	74.88		87.03		90.57	
LSD at 5%			5.84			

Hydrogen gas was started production from fourth day with all power supply at anode chamber 500 ml, the highest volume of Bio-H_2 112.83 cm^3 with power supply 0.8 V, which significant differences was found between this volume and other volumes. No significant differences were found between volume of Bio-H_2 98.16 cm^3 with power supply 0.6 V and the lowest volume of Bio-H_2 95.12 cm^3 (Table4). The bio-hydrogen production from domestic wastewater in MECs at the anode chamber 300 ml, 400 ml and 500 ml which indicates increasing the domestic wastewater degradation rat. From the thirteenth day the volume of bio-hydrogen started decreasing and stopped from fifteenth day.

The present results are in agreement with those reported by Ditzig *et al.,* (2007) who investigated the bio-hydrogen can be produced from domestic wastewater using MECs as bio-electrochemical reactors.

Table 4. The anode chamber 500 ml for Bio-H_2 (cm^3) production with power supply (0.4 V, 0.6 V and 0.8V) in MECs from domestic wastewater without bacterial addition.

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
2	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0
4	1.5	7.59	1.8	9.1	2.4	12.14
5	3.7	18.72	4.1	20.74	4.8	24.28
6	5.9	29.85	6.3	31.87	7.2	35.92
7	8.4	42.5	8.9	45.03	9.6	48.57
8	10.2	51.61	11	55.66	12	60.72
9	12	60.72	13.4	67.8	14.5	73.37
10	13.6	68.81	15	75.9	17.1	86.52
11	15.2	76.91	16.6	83.99	18.8	95.12
12	16.8	85	17.8	90.06	20.1	101.7
13	18	91.08	18.9	95.63	21.7	109.8
14	18.6	94.11	19.3	97.65	22.1	111.82
15	18.8	95.12	19.4	98.16	22.3	112.83
V H_2	95.12		89.16		112.83	
LSD at 5%			4.42			

Bio-hydrogen Production from domestic wastewater by *Enterobacter aerogenes* DSM 30053:

30 ml, 40 ml and 50 ml of bacterial culture of *Enterobacter aerogenes* DSM 30053 and 300 ml, 400 ml and 500 ml of domestic wastewater were added to anode chamber in MECs respectively and 300 ml, 400 ml and 500 ml of distilled water were added to cathode chamber . Fourth day with power supply 0.4 V and 0.6 V in MECs at the anode chamber 300 ml of domestic wastewater and fifth day with power supply 0.8 V, the hydrogen gas can be produced. Significant differences were found between the highest volume of Bio-H_2 138.13 cm^3 with power supply 0.4 V and other volumes of Bio-H_2 with power supply 0.6 V and 0.8 V . While the lowest volume of Bio-H_2 80.45 cm^3 with power supply 0.8 V. (Table 5).

These results are confirm with Lu *et al.,* (2012) they also used *Enterobacter aerogenes* for bio-hydrogen production from glucose as substrate in microbial electrolysis cells at low temperature.

Table 5. The anode chamber 300 ml for Bio-H_2 (cm^3) production with power supply (0.4 V, 0.6 V and 0.8V) in MECs from domestic wastewater by *Enterobacter aerogenes* DSM 30053.

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
2	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0
4	2.5	12.65	1.6	8.09	0	0.0
5	6.7	33.9	2.9	14.67	1.2	6.07
6	9.5	48.07	5.4	27.32	2.8	14.16
7	12.8	64.67	7.7	38.57	5.5	27.83
8	16.6	83.99	9.6	48.57	8.1	40.98
9	20.2	102.21	12.8	64.76	10.5	53.13
10	23.2	117.39	15.6	78.93	12.7	64.26
11	25	126.5	17.4	88.04	13.9	70.33
12	26.2	132.57	19	96.14	14.8	74.88
13	26.8	135.6	19.8	100.18	15.3	77.41
14	27.1	137.12	21.2	107.27	15.7	79.44
15	27.3	138.13	21.4	108.28	15.9	80.45
V H_2	138.13		108.28		80.45	
LSD at 5%			5.76			

In MEC the hydrogen gas production in cathode chamber started from second day with power supply 0.4 V and third day with power supply 0.6 V and 0.8 V onwards till fifteenth day at the anode chamber 400 ml. Significant differences were found between the highest volume of Bio-H_2 211.5 cm^3 with power supply 0.4 V and lowest volume of Bio-H_2 149.77 cm^3 with power supply 0.8 V. (Table 6).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 6. The anode chamber 400 ml for Bio-H_2 (cm^3) production with power supply (0.4 V, 0.6 V and 0.8V) in MECs from domestic wastewater by *Enterobacter aerogenes* DSM 30053.

Days / Power supply	0.4 V Burette reading (cm)	0.4 V Volume of Bio-H_2 collected (cm^3)	0.6 V Burette reading (cm)	0.6 V Volume of Bio-H_2 collected (cm^3)	0.8 V Burette reading (cm)	0.8 V Volume of Bio-H_2 collected (cm^3)
1	0	0.0	0	0.0	0	0.0
2	2.2	11.13	0	0.0	0	0.0
3	5.8	29.34	2.4	12.14	1.9	9.6
4	9.6	48.57	6.8	34.4	5.4	27.32
5	13.5	68.31	11	55.66	8.7	44.02
6	18	91.08	15.1	76.4	11.5	58.19
7	24.4	123.46	18.8	95.12	14.9	75.39
8	29.1	147.24	22.4	113.34	18.5	93.61
9	32.5	164.45	25.8	130.54	21.9	110.81
10	35.7	180.64	29	146.74	24.3	122.95
11	37.5	189.75	31.5	159.39	26.8	135.6
12	39.7	200.88	33.8	171.02	28.4	143.7
13	40.9	206.95	35.7	177.6	29.1	147.24
14	41.5	209.99	35.9	180.64	29.5	149.27
15	41.8	211.5	36.2	181.65	29.6	149.77
V H_2	211.5		181.65		149.77	
LSD at 5%			6.17			

Table 7 presents the volumes of bio-hydrogen production in MECs at the anode chamber 500 ml with power supply 0.4 V, 0.6 V and 0.8 V. The hydrogen gas was started production from second day with power supply 0.4 V and third day with power supply 0.6 V and 0.8 V. The highest volume of Bio-H_2 316.25 cm^3 with power supply 0.4 V, which were significant differences found between this volume and other volumes. The lowest volume of Bio-H_2 225.67 cm^3 was obtained with power supply 0.8 V. These results are confirm with Wang *et al.,* (2015) found that highest hydrogen yield and volume of bio-hydrogen were obtained with low power supply using molasses wastewater as substrate in MECs.

Table7. The anode chamber 500 ml for Bio-H_2 (cm^3) production with power supply (0.4 V, 0.6 V and 0.8V) in MECs from domestic wastewater by *Enterobacter aerogenes* DSM 30053.

Days / Power supply	0.4 V Burette reading (cm)	0.4 V Volume of Bio-H_2 collected (cm^3)	0.6 V Burette reading (cm)	0.6 V Volume of Bio-H_2 collected (cm^3)	0.8 V Burette reading (cm)	0.8 V Volume of Bio-H_2 collected (cm^3)
1	0	0.0	0	0.0	0	0.0
2	2.8	14.16	0	0.0	0	0.0
3	5.6	28.33	2.5	12.65	2.3	11.63
4	10.5	53.13	6.2	31.37	5.9	29.85
5	16.4	82.98	10.6	53.63	9.7	49.08
6	23.8	120.42	14	70.84	13.8	69.82
7	29.3	148.42	19.3	97.65	18.4	93.1
8	35.6	180.13	25.3	128.01	23.6	119.41
9	41.4	209.48	31.7	160.4	27.2	137.63
10	47.2	238.83	35.4	179.12	32	161.92
11	53.6	270.2	38.6	192.28	35.9	178.11
12	58.6	296.51	42.2	213.53	43.5	201.89
13	60.5	306.13	44.5	225.17	43.5	220.11
14	61.9	313.21	45.8	230.23	44.3	224.15
15	62.5	316.25	46.3	234.27	44.6	225.67
V H_2	316.25		234.27		225.67	
LSD at5%			6.45			

Production of Bio-H_2 Without and with Bacteria in MECs:

Evaluation of volume of Bio-H_2 production from domestic wastewater in the experiments with and without addition of bacteria while keeping all other condition the same. The highest volume of Bio-H_2 316 cm^3 production by *Enterobacter aerogenes* DSM 30053 at the anode chamber 500 ml with power supply 0.4 V. While the highest volume of Bio-H_2 production without addition of bacteria at the same chamber with power supply 0.8 V 112.83 cm^3 , which revealed significant positive relationship were found between the volume of Bio-H_2 production and addition of bacteria in MECs. But significant negative relationship increasing with power supply. The volume of Bio-H_2 production from domestic wastewater in MECs with and without addition of bacteria shown in Table (8). It is evident that the volume of Bio-H_2 evolved are more than doubled as an effect of adding bacteria. This results are agreement with Afify *et al.,* (2017) reported that the Bio-H2 production in MECs increased from second and third day till thirteenth day when inoculation with enteric bacteria (*E. coli* NRRL B-3008) which increasing the substrate degradation rate.

Table 8. Comparison of Bio-H_2 (cm^3) production in MECs without and with addition *Enterobacter aerogenes* DSM 30053 from domestic wastewater.

MECs	Power supply (V)	Without bacteria VH$_2$ (cm^3)	*Enterobacter aerogenes* DSM 30053 VH$_2$ (cm^3)
Anode	0.4 V	41.49	138.13
chamber	0.6 V	52.11	108.28
300 ml	0.8 V	54.64	80.45
Anode	0.4 V	74.88	211.5
chamber	0.6 V	87.03	181.65
400 ml	0.8 V	90.57	149.77
Anode	0.4 V	95.12	316
chamber	0.6 V	98.16	234.27
500 ml	0.8 V	112.83	225.67
LSD at 5%		7.92	16.13

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إستخدام بكتيريا الــ DSM 30053 *Enterobacter aerogenes* لإنتـاج الهيدروجين الحيوى بواسطة خلايا التحليل الكهربى الميكروبية من مياة الصرف الصحى

عايدة حافظ عفيفى[1] ، عمرو محمود عبد الجواد[2] و نبيل خضبرى عبد الرحمن[2]

[1] قسم الميكروبيولوجى – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .

[2] قسم خصوبة وميكروبيولوجيا الاراضى – مركز بحوث الصحراء – المطرية – القاهرة – مصر .

تهدف هذه الدراسة إلى استخدام خلايا التحليل الكهربى الميكروبية لإنتاج الهيدروجين الحيوى بواسطة ميكروب إنتروباكتر إيروجينيزمن مياة الصرف الصحى وذلك فى وجود ثلاثة احجام مختلفة من غرفة الأنود لخلية التحليل الكهربى الميكروبية وهى 300 مل ، و400 مل ، 500 ملليتر كما تم استخدام الجهد الكهربى الخارجى 0.4 فولت ، و 0.6 فولت ، 0.8 فولت كقوة داعمة لنقل الإلكترون ، فوجد ان أعلى إنتاج من الهيدروجبن الحيوى باستخدام مياه الصرف الصحى 112.28 سم ^{3} عند غرفة أنود 500 ملليتر وجهد كهربى خارجى 0.8 . بينما أعلى إنتاج من الهيدروجبن الحيوى عند إضافة بكتريا الــ DSM 30053 *Enterobacter aerogenes* إلى مياه الصرف الصحى هو 316 سم ^{3} عند غرفة أنود 500 ملليتر وجهد كهربى خارجى 0.4 .