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Evaluation of Pseudomonas aeruginosa ATCC 27853 for Bio-hydrogen production from El-Salam Canal Water.

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ABSTRACT

In this study EL-Salam canal water was used as substrate for Bio-hydrogen production in microbial electrolysis (MECs) by *Pseudomonas aeruginosa* ATCC 27853 and without addition of bacteria. The highest hydrogen yield was 31.18 % and the highest volume of Bio-H_2 production was 51.1 cm^3 from Salam canal water at the anode chamber 500 ml with power supply 0.8 V without addition of *Pseudomonas aeruginosa* ATCC 27853. While the highest hydrogen yield was 75.29% and the highest volume of Bio-H_2 was 148.25 cm^3 production by *Pseudomonas aeruginosa* ATCC 27853 from EL-Salam canal water at the anode chamber 500 ml with power supply 0.4 V.

Keywords: EL-Salam canal water, Microbial electrolysis cells, Bio-hydrogen, *Pseudomonas aeruginosa* ATCC 27853.

INTRODUCTION

Bacteria and cyanobacteria was used as biological process for Bio-hydrogen (Bio-H_2) production from biomass, wastewater and waste materials, biological process included fermentation methods , bio-photolysis and microbial electrolysis (MECs) . (Kotay and Das, 2008)

MECs are a new technology for bio-hydrogen (Bio-H_2) produced from organic matter. In MEC, at the positive (anode) chamber protons, electrons and CO_2 electrochemically active bacteria oxidize organic matter in wastewater. Electrons were transferred by bacteria from positive (anode) electrode to negative (cathode) electrode through a wire which connection between two electrodes. Protons were transferred to production chamber (negative chamber) through membrane or salt bridge. Hydrogen (Bio-H_2) produced in MEC at the negative (cathode) chamber when free protons were combined with electrons. MEC as bioreactor for bio-hydrogen production was needed power supply at lest 0.4V to complete production of Bio-hydrogen. (Liu *et al.,* 2005).

This study aims to El-Salam canal water was used as substrate in MEC as bioreactor for bio-hydrogen production and Hydrogen yield (%) with and without addition of *Pseudomonas aeruginosa* ATCC 27853. Studies the effect of volume of anode chamber (300, 400 and 500 ml) with power supply (0.4 V, 0.6 V and 0.8 V).

MATERIALS AND METHODS

Preparation of Bacteria and Substrate:

Pseudomonas aeruginosa ATCC 27853 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 (Afify, *et al*. 2017 a).

EL-Salam canal water was used as substrate for Bio-hydrogen. It was obtained from water-lifting No.5 – Baloza zone, North Sinai, Egypt. Drops of (1M) HCl acid was added to EL-Salam canal water the substrate for adjusted pH to 7 and 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 domestic 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).**

Volume of Bio-H_2 (cm^3) = length of burette reading (cm) × π 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

Hydrogen yield (%) and Bio-hydrogen produced from EL-Salam canal water without bacteria:

EL-Salam canal water was used at the substrate in MECs for Bio-hydrogen production. It filled in anode chamber (300, 400 and 500 ml) and distilled water in cathode chamber (300, 400 and 500 ml) respectively. Hydrogen gas was produced in cathode chamber started from sixth day at anode chamber 500 ml with power supply 0.6 V and 0.8 V to tenth day at the anode chamber 300 ml with power supply 0.4 V and 0.6 V. The highest volume of Bio-H_2 51.1 cm^3 production at the anode chamber 500 ml with power supply 0.8 V. The lowest volume of Bio-H_2 19.73 cm^3 production at the anode chamber 300 ml with power supply 0.4 V, which referring significant differences were found between other volumes of Bio-H_2 and this volume. No significant differences were found between all the volumes of Bio-H_2 production at the anode chamber 300 ml with all power supply.

EL-Salam canal water was used as substrate in MECs for production of volumes of bio-hydrogen at the anode chamber 300, 400 and 500 ml with power supply 0.4 V, 0.6 V and 0.8 V during fifteen days shown in Table (1). EL-Salam canal water is poor in organic matter contents compared to waste waters, therefore produced lowest volumes of bio-hydrogen production in MECs.

These results are consistent with those reported by Cucu *et al.,* (2013) who also used the River Sabar water (in Romania) as substrate in MEC and MFC for bio-hydrogen production and electrical generation. Also , Afify , *et al* . , (2017 b) reported that the hydrogen gas was significantly greater with power supply 0.4 V.

Table 1. Volume of Bio-H_2 (cm^3) produced at anode chamber 300, 400 and 500 ml from El-Salam canal water with power supply 0.4 V, 0.6 V and 0.8 V.

Days	Volume of Bio-H_2 collected (cm^3) at anode chamber 300 ml			Volume of Bio-H_2 collected (cm^3) at anode chamber 400 ml			Volume of Bio-H_2 collected (cm^3) at anode chamber 500 ml		
	0.4 V	0.6 V	0.8 V	0.4 V	0.6 V	0.8 V	0.4 V	0.6 V	0.8 V
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
V H_2	19.73	22.26	24.28	37.44	39.46	44.02	41.49	46.55	51.1

LSD at 5% = 3.48

Table 2 presents the hydrogen yield and volumes of Bio-H_2 production from EL-Salam canal water. The lowest hydrogen yield 14.05 % produced at the anode chamber 300 ml with power supply 0.4 V, which revealed significant differences were found between this yield and other yields at the anode chamber 400ml and 500ml. No significant differences were found between all the hydrogen yield production at the anode chamber 300 ml with all power supply. The highest hydrogen yield 31.18 % and the highest volume of Bio-H_2 51.1 cm^3 production at the anode chamber 500 ml with power supply 0.8 V.

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.

Hydrogen yield (%) and Bio-hydrogen produced by *Pseudomonas aeruginosa* ATCC 27853 from EL-Salam canal water:

MECs were filled with 30, 40 and 50 ml bacterial culture of *Pseudomonas aeruginosa* ATCC 27853 and EL-Salam canal water (300, 400 and 500 ml) in anode chamber respectively.

Table 2. Hydrogen yield (%) produced from EL-Salam canal water in MECs.

MECs	Power supply (V)	Δ COD (mg / L)	VH_2 (cm^3)	$^n H_2$ (mol)	^{n} th (mol)	YH_2 %
Anode	0.4 V	98	19.73	0.78	5.56	14.05
chamber	0.6 V	99	22.26	0.88	6.18	14.27
300 ml	0.8 V	99	24.28	0.96	6.18	15.56
Anode	0.4 V	101	27.44	1.48	6.31	23.52
chamber	0.6 V	101	39.46	1.56	6.31	24.79
400 ml	0.8 V	102	44.02	1.74	6.37	27.38
Anode	0.4 V	101	41.49	1.64	6.31	26.07
chamber	0.6 V	102	46.55	1.84	6.37	28.97
500 ml	0.8 V	104	51.1	2.02	6.5	31.18
LSD at 5%			3.48			1.9

Table (3) presents the volumes of Bio-H_2 (cm^3) produced at anode chamber 300, 400 and 500 ml by

Pseudomonas aeruginosa ATCC 27853 from El-Salam canal water with 0.4 V, 0.6 V and 0.8 V of power supply. The highest volume of Bio-H_2 148.25 cm^3 starting produced at third day by *Pseudomonas aeruginosa* ATCC 27853 in MECs at 500 ml of anode chamber with 0.4 V power supply obtained from EL-Salam canal water, which revealed significant differences were found between other volumes. No significant differences were found between the lowest volumes of Bio-H_2 52.11 cm^3 which starting produced from seventh day with 0.8 V at 300 ml of anode chamber and other volumes production at 300 ml of anode chamber. Significant negative relationship was observed between the volumes of Bio-H_2 and all power supply at different anode chamber volumes (300, 400 and 500 ml) of EL-Salam canal water. *Pseudomonas aeruginosa* ATCC 27853 able to degradation of organic matter in EL-Salam canal water as substrate and electron transfer to electrodes in MEC for production of bio-hydrogen.

These results are agreed with the results obtained by Nivedhan *et al.,*(2014), who stated that *Pseudomonas aeruginosa* able to production the highest volume of bio-hydrogen during 15 days from glycerol as substrate using MEC with power supply 0.4V to 0.8V.

Table 3. Volume of Bio-H_2 (cm^3) produced at anode chamber 300, 400 and 500 ml by *Pseudomonas aeruginosa* ATCC 27853 from El-Salam canal water with power supply 0.4 V, 0.6 V and 0.8 V.

Days	Volume of Bio-H_2 collected (cm^3) at anode chamber 300 ml			Volume of Bio-H_2 collected (cm^3) at anode chamber 400 ml			Volume of Bio-H_2 collected (cm^3) at anode chamber 500 ml		
	0.4 V	0.6 V	0.8 V	0.4 V	0.6 V	0.8 V	0.4 V	0.6 V	0.8 V
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	11.73	10.12	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	29.85	23.78	9.1
5	0.0	0.0	0.0	12.14	10.62	0.0	48.57	41.49	21.25
6	9.1	8.6	0.0	24.28	16.69	10.12	63.25	55.15	38.45
7	21.25	17.2	7.08	36.43	26.31	23.27	75.39	68.31	65.67
8	31.87	24.79	15.18	50.09	35.42	34.4	89.56	81.4	69.82
9	38.45	32.38	24.28	63.25	44.52	44.02	105.24	95.12	85
10	45.03	39.97	31.37	74.38	54.64	53.63	114.86	109.29	98.67
11	53.13	47.05	38.45	83.99	65.78	62.74	128.01	123.46	107.77
12	60.21	55.15	45.03	93.1	75.39	70.84	141.17	131.56	115.87
13	66.28	58.19	49.08	100.69	84.5	74.88	145.22	135.6	119.92
14	69.32	60.21	51.1	107.27	88.55	76.91	147.24	137.63	122.45
15	69.82	61.22	52.11	110.3	90.57	77.92	148.25	139.15	124.47
V H_2	69.82	61.22	52.11	110.3	90.57	77.92	148.25	139.15	124.47
LSD at 5% = 11.53									

The highest hydrogen yield 75.29 % obtained by *Pseudomonas aeruginosa* ATCC 27853 in MECs at the anode chamber 500 ml with power supply 0.4 V produced from EL-Salam canal water, which revealed significant differences were found between other yields. Table (4) presents the hydrogen yield and volumes of Bio-H_2 production from EL-Salam canal by *Pseudomonas aeruginosa* ATCC 27853. No significant differences were found between the lowest hydrogen yield 30.91 % and other yields production at the anode chamber 300 ml. Significant negative relationship was observed between the hydrogen yield and all power supply at different the anode chamber volumes (300, 400 and 500 ml) of EL-Salam canal water.

Also 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 electrolysis cell for bio-hydrogen production.

While the highest hydrogen yield 75.29 % and the highest volume of Bio-H_2 148.25 cm^3 production by *Pseudomonas aeruginosa* ATCC 27853 from EL-Salam canal water at the anode chamber 500 ml with power supply 0.4 V.

Table 4. Hydrogen yield (%) production by *Pseudomonas aeruginosa* ATCC 27853 from EL-Salam canal water in MECs

MECs	Power supply (V)	Δ COD (mg / L)	VH_2 (cm^3)	$^n H_2$ (mol)	$^n th$ (mol)	YH_2 %
Anode	0.4 V	110	69.82	2.77	6.87	40.29
chamber	0.6 V	109	61.22	2.42	6.81	35.65
300 ml	0.8 V	107	52.11	2.06	6.68	30.91
Anode	0.4 V	117	110.3	4.37	7.31	59.84
chamber	0.6 V	113	90.57	3.59	7.06	50.89
400 ml	0.8 V	111	77.92	3.09	6.93	44.57
Anode	0.4 V	125	148.25	5.88	7.81	75.29
chamber	0.6 V	123	139.15	5.52	7.68	71.82
500 ml	0.8 V	120	124.47	4.93	7.5	65.85
LSD at 5%			11.53			4.93

But the highest hydrogen yield 31.18 % and the highest volume of Bio-H_2 production 51.1 cm^3 from Salam canal water at the anode chamber 500 ml with power supply 0.8 V without addition of *Pseudomonas aeruginosa* ATCC 27853. Addition bacteria in MECs effect of increasing rate of hydrogen yield and volume of Bio-H_2 (Table 5). The significant positive relationships were found between the hydrogen yield or volume of Bio-H_2 production and addition of bacteria.

These results are agreed with the results obtained by Ujwal *et al.,* (2015) who stated that 22.9 % hydrogen gas was produced without bacteria from industrial wastewater and 45.8 % hydrogen gas produced when *Pseudomonas aeruginosa* was added.

Table 5. Comparison of Hydrogen yield (%) and volume of Bio-H_2 (cm^3) produced with and without *Pseudomonas aeruginosa* ATCC 27853 from EL-Salam canal water:

MECs	Power supply (V)	Without bacteria		*Pseudomonas aeruginosa* ATCC 27853	
		VH_2 (cm^3)	YH_2 %	VH_2 (cm^3)	YH_2 %
Anode chamber 300 ml	0.4 V	19.73	14.05	69.82	40.29
	0.6 V	22.26	14.27	61.22	35.65
	0.8 V	24.28	15.56	52.11	30.91
Anode chamber 400 ml	0.4 V	37.44	23.52	110.3	59.84
	0.6 V	39.46	24.79	90.57	50.89
	0.8 V	44.02	27.38	77.92	44.57
Anode chamber 500 ml	0.4 V	41.49	26.07	148.25	75.29
	0.6 V	46.55	28.97	139.15	71.82
	0.8 V	51.1	31.18	124.47	65.85
LSD at 5%		3.48	1.9	11.53	4.93

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تقييم بكتيريا *Pseudomonas aeruginosa* ATCC 27853 في إنتاج الهيدروجين الحيوى من مياه ترعة السلام

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فى هذه الدراسة تم إستخدام مياة ترعة السلام لإنتاج الهيدروجين الحيوى فى خلايا التحليل الكهربى الميكروبية بواسطة إضافة وكذلك عدم إضافة بكتيريا الـ *Pseudomonas aeruginosa* ATCC 27853 ، فوجد ان أعلى إنتاج من الهيدروجين الحيوى الناتج من استخدام مياه ترعة السلام 51.1 سم ^{3} بنسبة 31.8 % عند غرفة أنود 500 ملليتر وجهد كهربى خارجى 0.8 وذلك عند عدم إضافة البكتيريا . بينما أعلى إنتاج من الهيدروجين الحيوى الناتج عند إضافة بكتريا الـ *Pseudomonas aeruginosa* ATCC 27853 إلى مياه ترعة السلام هو 148.25 سم ^{3} بنسبة 75.29 % عند غرفة أنود 500 ملليتر وجهد كهربى خارجى 0.4 .