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BIO-HYDROGEN PRODUCTION IN MICROBIAL ELECTROLYSIS CELL USING WASTE WATER FROM SUGAR INDUSTRY

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ABSTRACT

Majority of energy is derived from fossil fuels, which are non-sustainable resources that may exhaust in near future. Urge to decrease the dependence on fossil fuels and increasing demand of energy by the society has motivated researchers to work on development of sustainable and green forms of energy. Hydrogen is considered as an alternative fuel due to its high combustion value and hence extensive research is currently being carried out on the development of hydrogen generation systems. Microbial Electrolysis Cell (MEC) is a promising new approach for biological hydrogen production from organic matter using microbes. Dual chambered MECs with a cation exchange membrane separating the chambers were fabricated and experiments were carried out to study the impact of parameters affecting the hydrogen production. Parameters such as electrode spacing and electrode potential were optimized while keeping the electrode material and electrode area constant. Waste water from sugar industry was used as substrate and impact of adding microbes externally was also studied. The gases produced during experiments with waste water from sugar industry as substrate contained 22.9% hydrogen. The volume of gases was doubled when *pseudomonas aeruginosa* was added externally while keeping all other parameters and conditions constant.

KEYWORDS: Microbial Electrolysis Cell, Bio-hydrogen, Industrial waste water, Renewable Energy, Pseudomonas aeruginosa

INTRODUCTION

Hydrogen gas is majorly produced from fossil fuels. Developing technologies for production of hydrogen from renewable energy sources such as biomass has gained momentum. Recent advances in energy production using organic matter include the generation of hydrogen in an MEC. An MEC is a promising new approach for biological hydrogen production from biodegradable organic matter using exo-electrogenic microbes. Though these systems show immense potential for green energy production, the utilization of these systems are still in infant stage in India.

In MEC electrochemically active microbes growing on the surface of the anode break down organic matter into CO2, electrons and protons. The electrons and protons travel through the external circuit and solution respectively and combine at the cathode to generate hydrogen. An externally supplied voltage is required because the coupled redox reaction is thermodynamically unfavorable. Less power is needed for the process than in water electrolysis because degradation of organic carbon in an MEC supplies part of the needed energy [1]. In MECs microorganisms play an important role in production of hydrogen. They interact with electrodes via electrons, catalysing oxidation reaction at the anode. Rate at which H_2 is released depends on how efficiently electrons get transferred from substrate to anode with the help of electrogens present in the anodic chamber. Pseudomonas aeruginosa is one such electrogen that can transfer electrons to anode in the presence of self-produced mediators [2]. Engineers prefer mixed cultures, rather than pure cultures for energy production from waste materials because mixed cultures utilize a greater variety of substrates. They are significantly more robust and easier to grow at large scales [3]. Various electrodes suitable for MECs and MFCs are listed in literature [4-6] among which carbonaceous electrode materials show high affinity to micro-organisms. Hence

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graphite plate is used as anode material and stainless steel plate is used as cathode material in this work.

In Dual chamber MECs membranes are placed between anode and cathode presumably to ensure high hydrogen concentrations and to eliminate hydrogen utilization by bacteria in anode chamber [7]. Nafion, CMI-7000 and Fumasep FKE are some commonly used cation exchange membranes [2]. CMI – 7000 cation exchanged membrane is used in this work.

In MEC hydrogen gas is formed at the cathode theoretically at minimum applied voltage of 0.135V [2]. In practice, due to electrode overpotential and ohmic resistance, more than 0.135V has to be applied to the MEC [8]. Most MECs are operated at applied voltages of 0.25-0.8V [9]. Applied voltages lower than 0.3 V may result in a low hydrogen-production rate and erratic system performance. Applied voltages above 1 V are not recommended because the electrical energy input is so large that the microbial electrolysis process becomes closer to a water electrolysis process [2]. In case of electrode surface area, cathode area is one of the limiting factors of hydrogen production in MECs [10]. In this work, anode to cathode surface area ratio of 1:2 is used.

Electrode spacing is the next important parameter affecting hydrogen production in MEC. Hydrogen production rate depends on current density and it is the internal resistance inside the cell that affects the current density. Internal resistance of cell decreases with decrease in electrode spacing, hence current density increases and there is an increase in hydrogen production. Through experiments it was found that with decrease in electrode spacing hydrogen production increases. But the closest electrode spacing do not necessarily produce the highest hydrogen production rates [11].

Among the various sources that can be used for energy generation in MECs, organic waste and wastewater are targeted first since they have potential to provide the greatest margins in profit and energy gain [2]. MECs have been tested with actual wastewater such as swine, domestic, and winery wastewater [12]. However no significant work is being carried out using waste water from sugar industry and hence the same is considered as substrate in this work.

MATERIALS AND METHODS

Construction of MEC

Four identical cells were fabricated that are cubical in shape as shown in *Figure 1*. Cubical cells were made of acrylic sheets of dimension 15cm x 15cm x 15cm (with 0.9cm thickness). CMI 7000 cation exchange membrane from Membranes International Inc. USA, was used to separate anode and cathode chambers. Lid is provided with two openings fitted with valves to facilitate gas collection. An arrangement was made to adjust the spacing between the electrodes and to hold the electrodes intact as shown in *Figure 2*. Silica gel was used as sealant.

Figure 1: *Cubical Microbial Electrolysis Cell made of acrylic material*

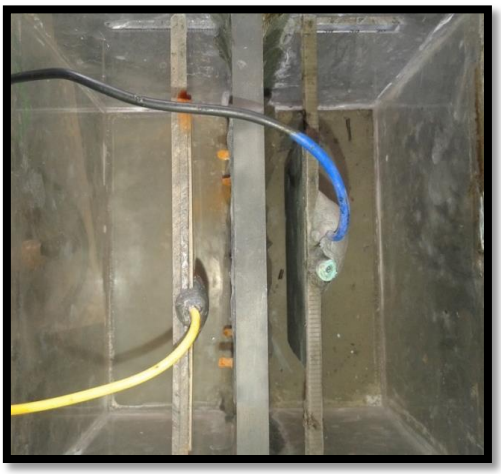

Figure 2: *Top view of MEC (with lid open) showing the electrodes with and arrangement to adjust spacing between the electrodes*

Based on literature and taking economic constraints into account stainless steel and graphite were selected as the electrode materials. Graphite plate of dimension 8cm x 8cm was used as anode and stainless steel plate of dimension 10.5cm x 12.2cm was used as cathode. A ratio of 1:2 was maintained between the electrode area of anode and cathode. Ferrous Ammonium Sulfate (CAS No. 7783-85-9, Sigma Aldrich), Potassium Dichromate (CAS. No. 7778-50-9, City Chemicals LLC) and Sulfuric acid

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(CAS. No. 7664-93-9, Sigma Aldrich) were used as received. A regulated Power Supply, 0-5V/500mA /DC/50Hz from Measurement Systems Pvt. Ltd was used to supply the required external voltage.

Analysis of wastewater

Turbidity, pH, Total Solids, Total Dissolved Solids, Total Suspended Solids and Chemical Oxygen Demand tests were carried out on waste water from sugar industry using standard test procedures and the following results were obtained as shown in Table 1.

Table 1: Analysis of waste water from a local sugar industry

TEST	Wastewater from Sugar Industry
pH	5.12
Turbidity (NTU)	340.00
Total solids (mg/Litre)	1728.00
Total dissolved solids (mg/Litre)	15722.00
Total suspended solids (mg/Litre)	1561.00
COD (mg/Litre)	10672.00

pH Maintenance

For the microbes to survive in the cell, the pH should be maintained at 7. Phosphate buffer (prepared by mixing 30.5 ml of 0.2 M dibasic sodium phosphate with 19.5 ml 0.2M monobasic sodium phosphate and diluting it to 100 ml using distilled water) was used to maintain the required pH. The pH was monitored using pH paper.

Microbial Culture

Culturing of microbes was carried out as part of the experiments, and the procedure mentioned in a work carried out by Rakesh et al [13] was followed.

Experimental Procedure

For carrying out experiments four MEC cells were used. First set of experiments were carried out by taking voltage and electrode spacing as variable parameters. Values of variable parameters were set according to factorial design. Anode chamber was filled with a liter of pre-analyzed waster water from sugar industry and cathode chamber was filled with distilled water. Phosphate buffer was added to anode until the pH was 7. A layer of grease was applied at the interface before covering the lid and vinyl tape was used to cover the joints to make the container airtight.

Figure 3: *MECs filled with waste water in anode chamber and distilled water in cathode chambers with electrodes connected to an external power supply*

Figure 4: *An arrangement to collect gases using burettes by downward displacement of water*

Regulated Power Supply was used to maintain required voltage at each cell. Anode was connected to positive terminal of RPS and cathode was connected to negative terminal of RPS through wire. Experimental setup can be seen in *Figure 3*. Gases produced in cathode chamber were collected in burettes by downward displacement of water as shown in *Figure 4*. Collected gases were analyzed for hydrogen content by Gas Chromatography.

RESULTS AND DISCUSSIONS

Experimental design

Six variables influencing the hydrogen production were identified based on literature.

1. Electrode Material

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2. Electrode Area
3. Substrate
4. Electrode Spacing
5. Electrode Potential
6. Microbes

The experiments were carried out in two sets. In both the sets only 2 parameters were varied keeping all other parameters constant.

Fisrt set of experiments

Fixed Parameters

1. Electrode Material
    a. Anode: Graphite
    b. Cathode: Stainless Steel
2. Electrode Area - Anode : Cathode :: 1 : 2
3. Substrate –Waste Water From Sugar Cane Industry

Variable Parameters

1. Electrode Spacing
2. Electrode Potential

According to experimental design each variable is varied between a fixed interval, a lowest (-1) level and a highest (+1) level. A linear relation between the variable and the output parameter is assumed on this interval which is required for the application of the experimental design. The lowest and highest levels of the variables are listed Table 2. An experimental design with 2 parameters yields $2^2 = 4$ experiments. Since 2 variables are used only first order interactions exist. Not just the effect of one variable can change the output parameter, but also the combined effect of the both variables.

Table 2: Variables and their levels

	Variable	-1	+1	Units
A	Electrode Spacing	2.2	4.0	cm
B	Electrode Potential	0.4	0.8	Volts

The first order interactions with their levels are specified in Table 3 and the experimental conditions are shown in .

Table 3: Experimental Design

	A	B	AB
1	-1	-1	1
a	1	-1	-1
b	-1	1	-1
ab	1	1	1

Table 4: Experimental design with actual values of variables

Experiment	Cells	Electrode Spacing (cm)	Electrode potential (V)
1	MEC-1	2.2	0.4
2	MEC-2	4.0	0.4
3	MEC-3	2.2	0.8
4	MEC-4	4.0	0.8

First set of experiments were carried out simultaneously in 4 cells MEC-1, MEC-2, MEC-3 and MEC-4 for 21 days. However gases were collected in MEC-4 only. The reason for no gases seems to be the least electrode spacing of 2.2cm in MEC-3, low electrode potential of 0.4V in MEC-2 and a combination of both less electrode spacing as well as low electrode potential in MEC-1.

According to the literature electrode spacing is specific to the size and geometry of the setup as well as the substrate. In general the hydrogen production increases with reduction in electrode spacing until an optimum value and the production decreases if the spacing is reduced further. In this case, 2.2 cm seems to be the value below which no hydrogen production takes place. In case of electrode potential the production of hydrogen increases with increase in potential and no hydrogen gas was produced at a voltage of 0.4 volts even though it is greater than the theoretical voltage requirement of 0.135V for hydrogen production in an MEC.

Details of gas collection in terms of volume are given in Table 5. The readings are based on the burette readings in cm and the corresponding volume in cubic centimetres.

Table 5: Volume of gases collected in MEC 4 with electrode spacing of 4cm and an electrode potential of 0.8v

Days	Burette reading (cm)	Volume of Gas Collected(cm^3)
1	0.0	0.0
2	0.0	0.0
3	6.0	42.4
4	8.4	59.3
5	12.8	90.4
6	20.0	141.3
8	25.0	176.6
9	29.0	204.9

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10	30.5	215.5
11	32.5	229.6
12	45.5	321.5
15	47.0	332.1
21	49.6	350.4

Figure 5 presents cumulative volume of gases collected in MEC-4 during experiment. Data shows that 90% of the gases were collected in first 12 days. The trend is linearly increasing during the first 11 days with a steep increase of 90cm^3 of gases (30% of total gases) just within a day. After 12 days only a small volume of gases are evolved until 21 days. At the end of the experiment these gases were analysed for hydrogen content and **22.9% of hydrogen** was found.

Second set of experiments

Fixed Parameters

Along with the electrode material and electrode area that were fixed in first set of experiments, electrode potential as well as the electrode spacing were also fixed in second set.

1. Electrode Material
   a. Anode: Graphite
   b. Cathode: Stainless Steel
2. Electrode Area - Anode : Cathode :: 1 : 2
3. Electrode Potential- 0.8 volts
4. Electrode Spacing - 4.0 cm

Along with these parameters, experiments were carried out by adding microbes *pseudomonas aeruginosa* externally.

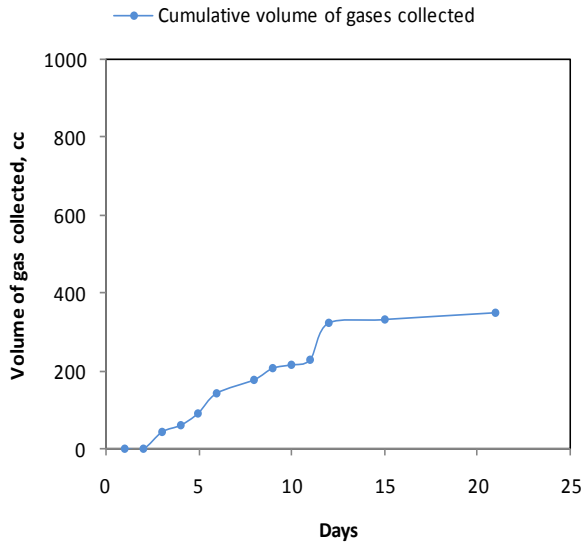

Figure 5: *Cumulative volume of gases collected in an MEC with a spacing of 4 cm and 0.8V potential difference between electrodes during 21 days of experiment*

The conditions maintained in experiment 5 were same as that of experiment 4 of set 1 except the addition of microbes. Experiment 5 can be comapred with Experiment 4 in order to study the impact of adding microbes externally. Volume of gases collected is shown in Table 6.

Table 6: Volume of gases collected in MEC with electrode spacing of 4cm, an electrode potential of 0.8v and external addition of pseudomonas aeruginosa

Day	Burette reading (cm)	Volume of Gas Collected (cm^3)
1	0	0.0
2	0	0.0
3	0	0.0
4	0	0.0
5	0	0.0
6	0	0.0
8	8	56.5
9	24	169.6
10	44	310.9
11	65	459.2
12	78.8	556.7
15	99.4	702.3
21	99.4	702.3

Figure 6 presents cumulative volume of gases collected in experiment 5. In this experiment a lag phase is observed during the first 6 days where no gases are evolved. This lag phase might be due to the addition of microbes externally, as these microbes need some time to get adjusted to the new environment. Soon after the lag phase there is a steep rise in volume of gases evolved. 80% of the total gases are evolved just within 4 days (from day 8 to day 12). Gases continued to evolve beyond day 12 until day 21.

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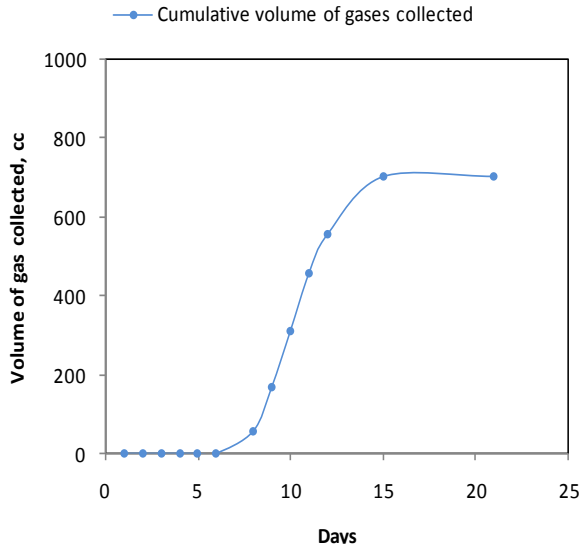

Figure 6: *Cumulative volume of gases collected in an MEC with a spacing of 4 cm and 0.8V potential difference between electrodes during 21 days of experiment in presence of microbes*

Evolution of gases in experiments with and without the addition of microbes while keeping all other condtions the same is shown in Table 7.

Table 7: Comparison of volume of gases collected in experiments wtih and without the addition of microbes externally

Day	Volume of gas collected (cm^3)	
	Without microbes	With microbes
1	0.0	0.0
2	0.0	0.0
3	42.4	0.0
4	59.3	0.0
5	90.4	0.0
6	141.3	0.0
8	176.6	56.5
9	204.9	169.6
10	215.5	310.9
11	229.6	459.2
12	321.5	556.7
15	332.1	702.3
21	350.4	702.3

Impact of adding microbes externally can be visualized in *Figure 7*. It is evident that the volume of gases evolved is doubled as an effect of adding microbes.

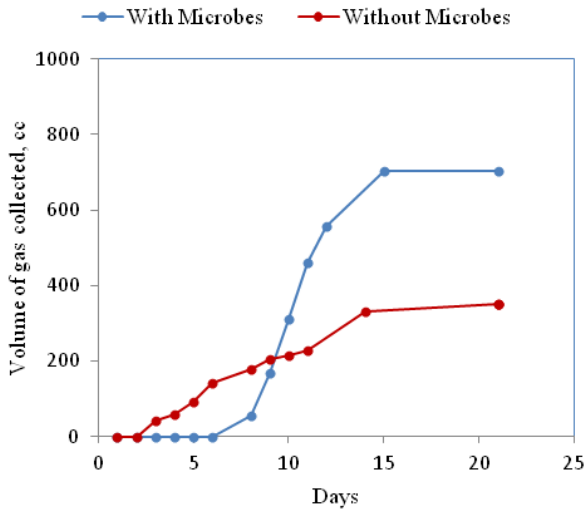

Figure 7: *Impact of adding pseudomonas aeruginosa externally on hydrogen generationin an MEC using waste water from sugar industry*

CONCLUSION

Based on the work carried out on MEC following conclusions can be drawn,

- In a MEC consisting of graphite anode and stainless steel cathode with a spacing of 4cm, a potential of 0.8 and waste water from sugar industry as substrate, **22.9% hydrogen** was produced without microbes.
- External addition of Microbes had a visible impact on the production of hydrogen. Volume of gases were doubled when Pseudomonas aeruginosa was added.

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