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Research Article

Biohydrogen Production through Single Chambered Microbial Electrolysis Cell (MEC)

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ABSTRACT

Biohydrogen production is a promising, clean, and renewable alternative to fossil fuels, and it plays a role in reducing greenhouse gas emissions and promoting energy security. Microbial Electrolysis Cells (MECs) utilize the metabolic capabilities of microorganisms to turn organic substrates into hydrogen gas, thus providing a much more efficient and sustainable energy solution. This study explores the potential of a membrane-less, single-chamber MEC for generating biohydrogen by utilizing stainless steel and copper wire as anodes. The results indicate a hydrogen production rate of 0.027 LSTP L_A^{-1} d^{-1} under non-limiting substrate conditions with an applied voltage of 1V. The standardization of electrode materials enhanced the process by limiting interference from methanogenic bacteria. Further optimization of electrode composition and operational conditions can improve scalability and efficiency, which makes MEC technology a feasible pathway for large-scale hydrogen production.

Keywords: Greenhouse Gas emissions, Microbial Electrolysis Cells (MECs), Methanogenic Bacteria, Large-Scale Hydrogen Production.

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INTRODUCTION

Bioenergy is the term used to describe a variety of energy sources that come from organic materials, including biomass, agricultural residues, organic waste, and crops grown specifically for energy. Bioenergy, which is dependent on biological processes, provides fossil fuels with low-carbon, renewable alternative, aiding in the efforts to achieve energy security and lower greenhouse gas emissions. The industrialized world needs clean fuels to fight greenhouse gas emissions.

As a carbon-neutral substitute, biohydrogen has the potential to significantly lessen reliance on finite fossil fuel supplies and mitigate the effects of climate change. The process of producing biohydrogen involves using different microorganisms' metabolic capacities to transform organic substrates into hydrogen gas through biological processes. By using organic feedstocks like biomass. Agricultural residues, and organic waste, this method not only offers a sustainable way to produce energy but also a way to address waste management issues.

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The term 'Hydrogen Economy' was first coined by Prof. John Bockris during a talk he gave in 1970 at the General Motors Technical Center.[1] Hydrogen is believed to be one of the most abundant elements in the universe. The heat release per unit mass of hydrogen was found to be three times more than that of gasoline. Furthermore, it is a clean, renewable energy source that generates water vapor alone on combustion.[8] Moreover, production of hydrogen via various renewable energy production technologies is based on energy production technologies like wind, solar, and nuclear energy. Hydrogen is produced by the technologies dependent on biomass, and this includes biomass decomposition through microbial, chemical, and electrolytic action.[2] Biohydrogen can be generated through a variety of feedstocks through thermochemical technologies like pyrolysis, steam reforming of biobased oils, simple gasification, supercritical gasification of water, steam gasification, and biological processes.

The biological processes are environmentally friendly; they are an efficient method of producing hydrogen. Numerous blue-green algae species, bacteria, higher plants and green algae are known to produce hydrogen and oxygen at room temperature and pressure. Through this process, renewable energy is produced from the most abundant resources, like solar and water energy, with its enormous potential. Biohydrogen could greatly outperform other hydrogen production technologies that have garnered international attention and rely on om fossil fuels.[3]

Electrohydrogenesis is a newly established electrolysis method that is primarily used for the direct conversion of biodegradable material into hydrogen using modified microbial fuel cells. In an MFC, exoelectrogens induce the oxidation of organic matter and causes the electron transfer to the anode. The electrons move under the influence of external resistance and join the protons at the cathode and oxygen to generate water. A Microbial Electrolysis Cell (MEC) and an MFC operate similarly, other than the fact that the cathode is isolated to exclude oxygen, and an external voltage is added to the circuit. An external voltage is necessary as an acetate substrate cannot spontaneously generate hydrogen under standard conditions.[4]

Microbial Electrolysis has been found to offer a valuable and cutting-edge route. The concept of Microbial Electrolysis Cells (MEC) was first presented in 2005. The transformation to an MEC from an MFC needs two changes. The first change is to isolate oxidants that are part of the cathode, which in turn results in the electrons being donated to hydrogen ions, thereby generating hydrogen at the cathode. The second change is to supply a specific amount of electrical energy to sufficiently generate a negative potential at the cathode to generate hydrogen. The electrolyte in the anode must possess the culture medium and certain microorganisms that are essential for their development. Certain microorganisms spontaneously occupy the anode's surface to form an electroactive biofilm, which acts as an electro-catalyst. The biofilm creates microbial oxidation in the anode at a large variety of low-cost carbon compounds. Microbial Electrolysis Cell (MEC) is a novel and promising approach for generating hydrogen from organic matter in a cost effective, sustainable, and renewable manner. Although, at present, most hydrogen production technologies utilize non-renewable fossil fuels like natural gas.[5] The main advantage of MEC when compared to abiotic water electrolysis is that the oxidation of water is substituted by the oxidation of organic compounds, which can take place at significantly lower redox potential. A new MEC is intended to boost this process energy recovery while lowering membrane losses. Several features, including anodes treated with ammonia, high surface area graphite brush anode, and short electrode distance, were tested in a novel MEC design without the presence of a membrane to decrease potential losses related to the membrane and increase the energy recovery of this process. Membrane less single chamber MEC can function without membranes, simplifying the architecture and lowering capital expenses.[6]

Initial demonstrations of hydrogen production using microbial electrolysis were executed under conditions that were suboptimal where low volumetric efficiencies of hydrogen production were achieved. Given that around 1 mol of hydrogen can be expected if anodiphilic biomass density is similar to that of a high-rate anaerobic reactor.[7]

This study presents the effort in developing a high-rate continuous flow of MEC with a suitable anode, cathode, substrate concentration and applied voltage.

AIM

This study aims to assess the potential of a membrane-less, single-chamber Microbial Electrolysis Cell (MEC) for biohydrogen generation by utilizing stainless steel and copper wire as anodes. The study looks to optimize the materials of electrodes to increase the yield of hydrogen while decreasing interference from methanogenic bacteria, which in turn contributed to the scalability and efficiency of MEC technology for clean and sustainable hydrogen production.

MATERIALS AND METHODS

Material required

Anode-Graphite plate and copper wire.

Cathode- Stainless steel mesh and stainless-steel wire (Figure 1).

Glass bottle-500 mL.

Standardization of Microbial Electrolysis Cell

Microbial Electrolysis Cells (MECs) are bioelectrochemical systems that are generally used for the conversion of electrochemically active bacteria into valuable by-products like hydrogen or other organic matter.[8] They have multiple beneficial applications in the treatment of wastewater[9] and energy production through sustainable means.[10] However, without the proper standardization of microbial electrolysis cells, it affects the robustness of the studies conducted that are related to it making it almost impossible to reproduce the results across different studies. Therefore, proper standardization ensures that the studies conducted in a research facility can be used for its implementation in an industrial setting.[11]

The main principle behind microbial electrolysis cells is bioelectrochemical conversion,[12] where at the positively charged anode, electrochemically active bacteria oxidize the organic substrates that are made available for them.[13] As a result of this oxidation at the anode, both protons and electrons are released. The electrons that are released due to the oxidation of the substrate move towards the negatively charged cathode due to the externally applied voltage.[14]

$$\text{Substrate}+H_2O \rightarrow CO_2+H^+ + e^- \text{ (anode)}$$

This voltage that is applied to the MECs typically ranges between 0.2-1.0 V.[15] On the other hand, the protons move to the cathode either through the electrolyte solution or a proton exchange membrane.[16] After the migration of both the protons and electrons to the negatively charged cathode, they combine

to generate hydrogen gas.[17] In this process, the generation of hydrogen gas is conducted in a more efficient manner.[18]

$$2H^{+}+2e^{-}\rightarrow H_{2}\ (\text{cathode})$$

MECs can be configured into different designs, and they are done so depending on the rate of hydrogen production and its efficiency. Usually, MECs are configured either into a single-chamber system or a double-chamber system.[19] In this case, certain metrics like the size of the chamber and materials used need to be properly standardized. Performing this step is critical as it ensures that the data like the rates of hydrogen production and hydrogen production efficiencies across multiple different studies are comparable.[20]

In a single-chamber system, both the anode and cathode are accommodated in a single chamber without the presence of a physical barrier like a proton exchange membrane separating them.[21] Since the electrodes are present in a singular chamber, this leads to a decreased internal resistance, which in turn results in a higher rate of hydrogen production.[22]

Additionally, the absence of another physical barrier like a proton exchange membrane leads to a much simpler design of the reactor and reduced costs.[23] However, the presence of the anode and cathode in a single chamber also comes with certain disadvantages. Since multiple gases are generated in this process, there is a possibility that these gases would mix with each other. In this case, both carbon dioxide and hydrogen are generated in different stages of the process. Therefore, there is a high chance that the hydrogen generated would mix with carbon dioxide, thereby decreasing the purity of the hydrogen gas that is generated.[24]

In a double-chamber system, both the anode and cathode are present in separate chambers, and they are separated by a physical barrier like a proton exchange membrane. Since both the electrodes are present in separate chambers, the likelihood of gases interacting with each other is low.

Here, the gas from the cathode is separated, therefore, the possibility of the hydrogen gas mixing with the other gases is low, thereby reducing the contamination and increasing the purity of the hydrogen gas generated.[25] However, the presence of a proton exchange membrane leads to a much more complex design of the reactor and increased costs.[26] Furthermore, due to the presence of two chambers in this process, maintaining a stable pH across both chambers may prove to be a difficult task.[13]

Choosing the right material for an electrode is important as it is directly linked to the performance of the MECs. Electrode material also directly influences various performance metrics like the efficiency of the transfer of electrons across the circuit and the stability of the whole process. The material of the electrode is selected based on what is needed for that study.[27] Carbon based electrodes like graphite and carbon fiber are preferred mainly due to their high conductivity and increased chemical stability. High conductivity ensures that the electron transfer between the cathode and an anode occurs in an efficient manner.[28]

Metallic electrodes like titanium and stainless steel are selected for the reactor primarily due to their robustness and consistency across various different conditions. High robustness ensures that the results from the study can be reproduced under varying conditions.[29] However, additional materials like polymers are integrated with the metallic electrodes to improve other aspects of the electrode like conductivity which may be lacking in metallic electrodes. Recent advances have shown the integration of components like carbon nanotubes to the electrodes to enhance the conductivity and surface area of the electrode.[30] This is because an electrode with a higher surface area is directly linked to an increased colonization of electrochemically active bacteria, thereby resulting in an increased rate of electron transfer across the electrodes.[31]

It is important to supply the appropriate amount of external voltage to the MECs, as the external voltage applied directly provides the energy to the MECs that is necessary for the generation of hydrogen gas. Depending on various factors like the design of the reactor and microbes used, the external voltage applied to the MECs typically ranges from 0.2 V to 1.0 V.[15]

Supplying the right voltage is critical as too little voltage can lead to inadequate impetus for hydrogen production. On the other hand, too much voltage can lead to unwanted energy losses and generation of heat. Therefore, a standardized voltage range needs to be established, and the voltage can be selected based on factors such as the design of the reactor as well as the microbial performance.[25]

Another factor that directly influences the generation of hydrogen gas is the substrate. The substrate directly influences various factors like the generation of electrons and microbial activity. Choosing the right amount of substrate is critical as it is directly

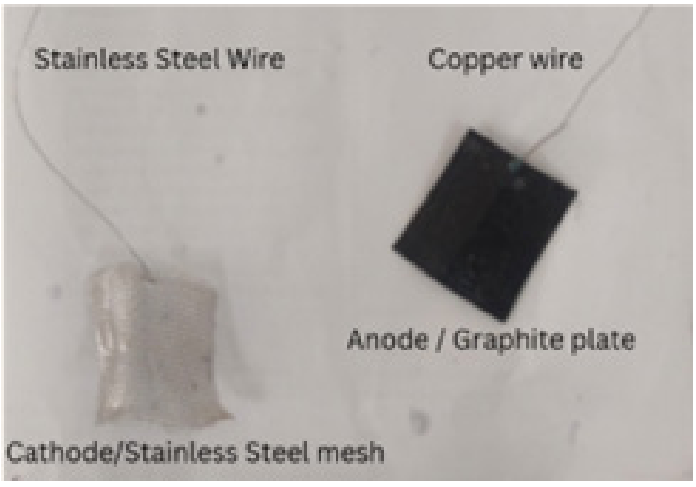

Figure 1: Standardization of the electrodes.

linked to hydrogen production and the improper amount can negatively impact the production of hydrogen gas.[32]

High substrate concentration can lead to increased microbial activity, which in turn leads to an increase in the generation of electrons. However, there is a possibility that the high substrate concentration can lead to it exceeding the metabolic capacity of the microbes.[25] On the other hand, low substrate concentration can result in inadequate energy for the growth of microbes, thus this will hinder electron generation and lead to a decreased production of hydrogen.[13]

Furthermore, selecting the type of substrate is equally important as it can directly impact the generation of electrons and hydrogen production. Simple organic substrates like glucose are a type of substrate used in MECs, where they are usually composed of simple and biodegradable compounds. As a result, they are quickly metabolized by the microbes and lead to an increased hydrogen production.[33] However, this is not feasible in the real world as it is rare to gain access to substrates with simple compounds.[13] Substrates composed of complex wastewater are another type of substrate used in MECs. These substrates can be obtained from sources like industrial wastewater and agricultural crop residues.[8] These substrates are more complex and possess more diverse organic compounds. Therefore, this leads to the microbes degrading the substrate more slowly, and this in turn leads to a decrease in hydrogen production.[34]

The anode was heat-treated at 121°C for 15 min. The anode (Figure 2) was left in 30% HCl overnight. The cathode (Figure 3) was rinsed in the milli Q Water 3 times and autoclaved. The chamber was left open for 30 min. The MEC mode was done in the sterilized condition and the medium's pH was maintained at 6 for the inhibition of the methanogenic bacteria growth in the cell. The opening of the chamber was sealed with aluminium foil to resist photolytic fermentation.

Methanogens bacteria are readily available to utilize biomass or the substrate to produce the methane gas. Hence to inhibit the growth of such bacteria can be achieved by optimization of the electrodes and the MEC chamber (Figure 4). The *Alcaligenes faecalis* was isolated from the aquifer sediment; the pure culture was used for the MEC setup (Figure 5). In this study the bacteria were cultured aerobically in the nutrient broth. The glass bottle MECs were loaded with a medium solution (300 mL) containing acetic acid of 0.3 mL, Sodium dihydrogen phosphate of 3.06 g, disodium hydrogen phosphate of 10.14 g. The pH of the solution was adjusted to 6 with the help of HCl. The MEC was secured with a cap and aluminium foil and then autoclaved. The MECs were then inoculated with the organism and operated at an applied voltage of 1V. All the experiments were operated in a semi-continuous mode whilst maintaining a temperature of 30°C-37°C using a temperature-controlled chamber. The chambers were all surrounded with aluminium foil to eradicate the possibility of generating unwanted hydrogen due to photo fermentation.

Variation in Voltage with the constant substrate concentration

In this study the bacteria were cultured aerobically in the nutrient broth. The glass bottle MECs were loaded with a medium solution (300 mL) which consists of 0.3 mL acetic acid, 3.06 g sodium dihydrogen phosphate, and 10.14 g disodium hydrogen phosphate. The MEC was sealed with the cap and with aluminium foil and autoclaved. The MECs were then inoculated with the organism and operated at a voltage of 0.6, 0.8, 1, 2 and 9V. All the experiments were conducted in a semi-continuous mode whilst maintaining a temperature of 30°C-37°C using a temperature-controlled chamber. The chambers were all surrounded with aluminium foil to eradicate the possibility of generating unwanted hydrogen due to photo fermentation.

Figure 2: Anode (graphite plate).

Figure 3: Cathode (Stainless Steel mesh).

Table 1: MEC performance of hydrogen yield with the Electrode (copper wire).

Substrate variation	Voltage variation	Δ_t=t-t_0	W=(S_0-S_t)	CE value (L_{STP} L_A^{-1} d^{-1})
0.3	0.6	24	0.06	0.004
0.3	0.8	24	0.06	0.005
0.3	1	91	0.06	0.027
0.3	9	4	0.06	0.010
0.5	1	119	0.012	0.007
0.5	2	24	0.012	0.002

Table 2: MEC performance of hydrogen yield with the Electrode (Stainless steel).

Substrate variation	Voltage variation	Δ_t=t-t_0	W=(S_0-S_t)	CE value (L_{STP} L_A^{-1} d^{-1})
0.3	2	48	0.06	0.02
0.5	2	0	0	0

Variation in concentration substrate along with the voltage

In this study the bacteria were cultured aerobically in the nutrient broth. The glass bottle MECs were loaded with a medium solution (300 mL) which consists of 0.5 mL acetic acid, 3.06 g sodium dihydrogen phosphate, and 10.14 g disodium hydrogen phosphate. The MEC was sealed with a cap and with aluminium foil and autoclaved. The MECs were then inoculated with the organism and operated at an applied voltage of 1 and 2V. All the experiments were conducted in a semi-continuous mode whilst maintaining a temperature of 30°C-37°C using a temperature-controlled chamber. The chambers were all surrounded with aluminium foil to eradicate the possibility of generating unwanted hydrogen due to photo fermentation.

Variation in the electrode

In this study the bacteria were cultured aerobically in the nutrient broth. The glass bottle MECs were loaded with a medium solution (300 mL) which consists of 0.3 mL acetic acid, 3.06 g sodium dihydrogen phosphate, and 10.14 g disodium hydrogen phosphate. The MEC was sealed with a cap and with aluminium foil and autoclaved. The MECs were then inoculated with the organism and operated at an applied voltage of 2V. All the experiments were conducted in a semi-continuous mode whilst maintaining a temperature of 30°C-37°C using a temperature-controlled chamber. The chambers were all surrounded with aluminium foil to eradicate the possibility of generating unwanted hydrogen due to photo fermentation.

Variation in the electrode along with the different substrate concentration

In this study, the bacteria were cultured aerobically in the nutrient broth. The glass bottle MECs were loaded with a medium solution (300 mL) which consists of 0.5 mL acetic acid, 3.06 g sodium dihydrogen phosphate, and 10.14 g disodium hydrogen phosphate. The MEC was sealed with a cap and with aluminium foil and autoclaved. The MECs were then inoculated with the organism and operated at an applied voltage of 2V. The experiments were operated in a semi-continuous mode. All the experiments were conducted in a semi-continuous mode whilst maintaining a temperature of 30°C-37°C using a temperature-controlled chamber. The chambers were all surrounded with aluminium foil to eradicate the possibility of generating unwanted hydrogen due to photo fermentation.

MEC calculation

An Adjustable DC power supply was utilized to maintain the voltage at the required setpoint.[7]

In MEC mode, voltage scan was performed by changing the applied voltage between 0.6, 1, 2, 4, 9V (Figure 6) and measuring the resulting current. Hydrogen yield was calculated over the time interval $\Delta t = t - t_0$ as follows:

$$Y_{H_2} = \frac{\left(\frac{P \times F_{H_2} \Delta t}{RT}\right)}{\frac{w}{M}}$$

Where p is the pressure (p=1atm); F_{H_2} is the hydrogen production rate; R is the ideal gas constant; T is the temperature (T=310.15 K); M is the substrate consumed for hydrogen production (g).

The apparent Coulombic Efficiency (CE) of hydrogen production was estimated as:

$$CE = \frac{I \times \Delta t \times M}{F \times n \times W}$$

Where I is the average current; Δt is the time interval during which current was measured; F is Faraday's constant, 96489 ($Cmol^{-1}$) and n is the number of electrons transferred per mol of the substrate oxidized into CO_2 (n=8 for acetate) (Table 2).

Figure 4: Single Chambered MEC.

Figure 5: Simple streak of the *Alcaligenes faecalis*.

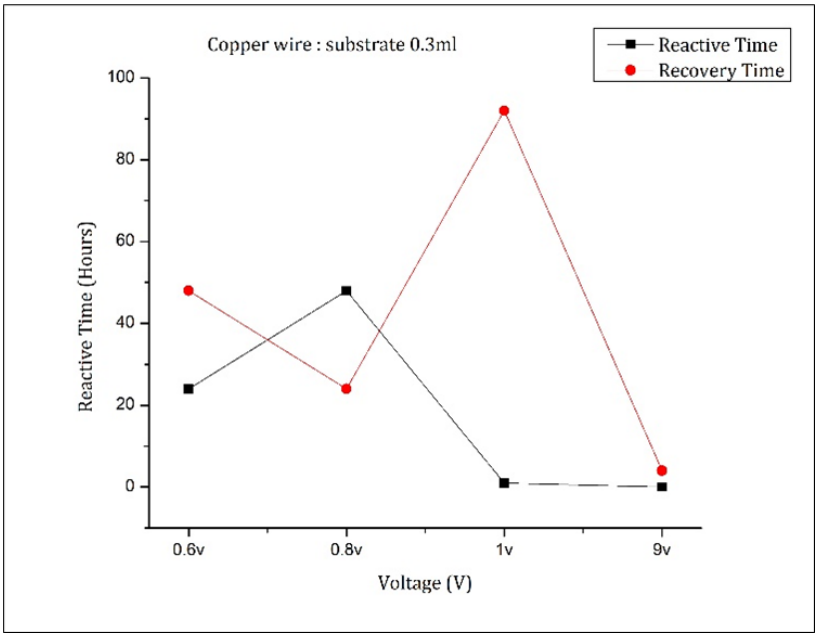

Figure 6: Reactant and Recovery time of the MEC with the electrode of copper wire and graphite sheet at the concentration of substrate 0.3 mL and different voltages of 0.6V, 0.8V, 1V, 9V.

RESULTS AND DISCUSSION

At various concentrations of substrate and different voltages, the experiment was carried out. The electrode was made of copper wire and graphite plate as the anode and stainless steel as a standard cathode at all the MEC modes. The voltage vs time were plotted (Figure 6) and on analysis, it reveals the reactive and recovery time of the electrode at different voltages. The material shows good reactive time for the copper electrode suggesting favorable electron transfer kinetics. The high recovery time of hydrogen production was observed at the concentration of substrate 0.3 mL and 1V (Figure 7). This implies the sustainability of the copper wire as the electrode material in the 1V was a desired outcome.

The sustainable electrode was observed to be platinum wire in the.[4] The platinum wire was used as a standard anode material. The production of hydrogen was observed to be 0.05 m^3 H_2/m^3d with an applied voltage of E_{ap}=0.4V. The variation in the substrate concentration and voltages makes a huge difference in the rate of hydrogen production. The standardization of the substrate level has proved to increase the hydrogen yield. The applied voltage has the potential to increase hydrogen production. The amount used as a standard substrate concentration of 0.3 mL was utilized rapidly in the applied voltage of 1V (Figure 8). This study's outcome has been clear that the hydrogen produced in the experiment was enhanced by the utilization of substrate at the 1V proving that the hydrogen yield depends upon the substrate concentration and the applied voltage.

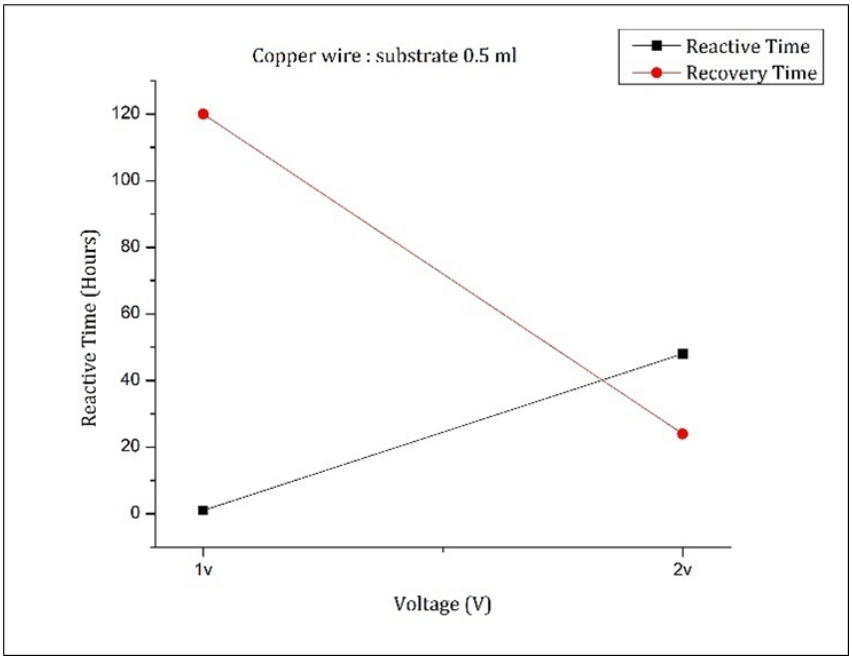

Figure 7: Reactant and Recovery time of the MEC with the electrode of copper wire and graphite sheet at the concentration of substrate 0.5 mL and different voltage of 1V, 2V.

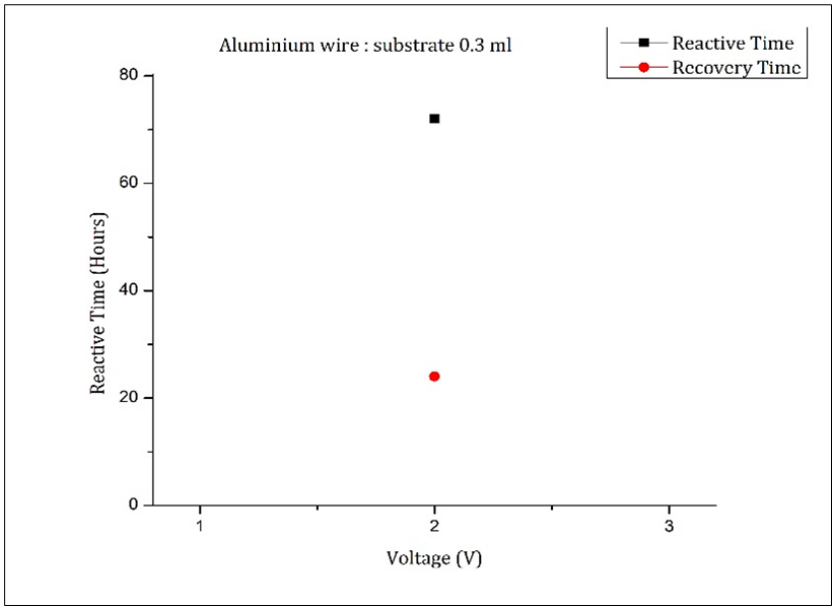

Figure 8: Reactant and Recovery time of the MEC with the electrode of Stainless Steel and graphite plate at the concentration of substrate 0.3 mL and voltage of 2V.

COULOMB'S LAW CALCULATION ANALYSIS

The value of the CE is high at 0.3 mL of substrate concentration at 1V (Table 1). The value was 0.027 L_{STP} L_{A}^{-1} d^{-1} in the electrode of copper wire.

The hydrogen production reached 6.3 L_{STP} L_{A}^{-1} d^{-1} at the substrate concentration of 450 mg/L^{-1} at 1.15V. The different yields of hydrogen were observed due to the difference in the anode and substrate levels in the conducted experiment. Throughout the tests, the rate of acetate removal was proportional to applied voltage confirming that acetic acid was consumed by anodophilic microorganisms. Calculations of acetate recovery using material balance showed a recovery of 40-90%. Significant hydrogen losses through the opening of the chamber were observed. Hence a coulombic efficiency in the range of 80-100% was reported for a single chamber MEC setup. The comparison of the Coulomb's law shows that the rate of the hydrogen yield was directly proportional to the acetate recovery.[7]

CONCLUSION

This study demonstrated a high rate of hydrogen production in a membrane-less MEC with copper wire and stainless steel as an anode. The hydrogen production was 0.027 L_{STP} L_{A}^{-1} d^{-1} in the electrode of copper wire. A volumetric hydrogen production rate was achieved under the substrate non-limiting conditions at an applied voltage of 1V. The standardization of the electrodes enhanced the MEC where the substrate concentration was directly utilized by the anodiphilic bacteria without the hindrance of methanogenic bacteria and other factors. The feasibility of this electrode material and the substrate at the small scale can be further developed to a larger scale and increase the volumetric hydrogen production rate by the optimization of the electrode materials and the substrate concentration. A decrease the methane production can be expected through the optimization of electrode materials, operational conditions, and the use of biocathodes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MEC: Microbial Electrolysis Cells; **MFC:** Microbial Fuel Cells; **CE:** Coulombic Efficiency; **HCl:** Hydrochloric Acid; **p:** Pressure; F_{H_2}**:** Hydrogen Production Rate; **R:** Ideal Gas Constant; **T:** Temperature; **M:** Substrate consumed for hydrogen production; **I:** Average Current; **Δt:** Time interval during which current was measured; **F:** Faraday's Constant; **DC:** Direct Current.

SUMMARY

The potential of a membrane-less, single-chamber Microbial Electrolysis Cell (MEC) for biohydrogen production as a sustainable alternative to fossil fuels was explored. Using stainless steel and copper wire as anodes, the system achieved a hydrogen production rate of 0.027 LSTP L_{A}^{-1} d^{-1} under non-limiting substrate conditions with an applied voltage of 1V. The standardization of the electrode materials reduced interference from methanogenic bacteria, which subsequently improved process efficiency. These findings indicate the MEC technology's potential for renewable and scalable hydrogen production, with further optimization of parameters like electrode composition and operational conditions necessary for large-scale application.

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