Design and optimization of a single-chambered membrane-less reactor for microbial electrosynthesis (MES) of acetic acid from CO_2

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Certificate of Examination

This is to certify that the dissertation titled "Design and optimization of a single-chambered membrane-less reactor for microbial electrosynthesis (MES) of acetic acid from CO_2" submitted by Mr. Prathamesh B. Pol (Reg. No. MS16094) for the partial fulfillment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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(Supervisor)

Dated: 26 May, 2021

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Sunil A. Patil at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Prathamesh B Pol

(Candidate)

Dated: 26 May, 2021

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Sunil A. Patil

(Supervisor)

Dated: 26 May, 2021

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Notations

BESs	Bioelectrochemical Systems
MES	Microbial Electrosynthesis
PEM	Proton Exchange Membrane
CCU	Carbon Capture and Utilization
CCS	Carbon Capture and Storage
CA	Chronoamperometry
CV	Cyclic Voltammetry
ET	Electron Transfer
EET	Extracellular Electron Transfer
DET	Direct electron transfer
GHG	Greenhouse Gas

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✧ Abstract

Microbial electrosynthesis (MES) is an exciting and dynamic research area at the nexus of microbiology, electrochemistry, material sciences, reactor engineering, and environmental sciences. In MES, microbes having the ability to fix CO_2 via the Wood-Ljungdahl pathway are used as biocatalysts for the electricity-driven conversion of CO_2 into chemical compounds (e.g. acetic acid, methane, butyric acid, etc.). Considerable advancements have occurred in MES technology through intense research focus on microbial catalysts, electrode materials, and process control variables. However, it is still far from practical application due to high ohmic losses, high cost, low gas-liquid mass transfer, high over-potential, and operational complexities associated with its routinely used two-chambered reactor design. In traditional two-chambered 'H-type' reactors, anode and cathode chambers are separated by a proton-exchange membrane (PEM). PEM allows the passage of protons and minimizes O_2 transfer to the cathode chamber. As microbes present in the cathode chamber are anaerobic, O_2 contamination can adversely affect their growth. However, PEM is expensive, sensitive, and requires pretreatment, thus hindering the scaling up of the technology. Also, due to its complexities, expert handling is required for long-term operation. Moreover, PEM restricts the free flow of protons between the electrodes, which adds to the ohmic losses. The distance between electrodes in a two-chambered reactor is more, which is another cause of higher ohmic losses. Hence, to pave the way for MES to industrial application, there is a need to investigate improved reactor designs that cater to both biological and electrochemical requirements.

My thesis work focused on designing and testing a single-chambered membrane-less reactor with a unique cathode and anode placement strategy to address some of the issues associated with conventional two-chambered MES reactors. We demonstrate MES of acetic acid from CO_2 in a proof-of-concept customized design using a mixed microbial

inoculum source dominated by *Acetobacterium* sp. At an applied cathode potential of -1.2 V *vs.* Ag/AgCl, about 0.6 ± 0.4 g/L acetic acid was produced at volumetric and cathode surface area-based production rates of 0.06 ± 0.04 g/L/d and 20.0 ± 14. g/m^2/d, respectively. About 84 ± 34% electrons were recovered in acetic acid. The O_2 produced at the anode was flushed out of the reactor by continuous N_2 sparging at 25ml/min to maintain anaerobic conditions. The E_{cell} was -2.6 V vs Ag/AgCl, which is lower than the conventional two-chambered MES reactors (mostly > -3 V vs Ag/AgCl). The bioproduction at a low applied voltage means a low energy input and high energy efficiency.

This study demonstrates that the bio-conversion of CO_2 into acetate via MES can be achieved by developing and optimizing a single-chambered membrane-less reactor. Some operational issues need to be addressed through further work. For instance, the trace amount of O_2 was detected in the headspace of the reactor. It reduces production efficiency as the microbes used for biocatalysis are anaerobic. Hence, by further upgradation like narrow anode opening and better N_2 sparger, O_2 contamination can be avoided. Pressurizing headspace with excess CO_2 will reduce O_2 diffusion from the anode chamber to the headspace. A high acetic acid production rate with higher coulombic efficiency can be achieved by optimizing operational parameters like electrode potential, electrode material and size, and improved CO_2 solubility.

1. Introduction

The burning of fossil fuels and rapid scale industrialization during the past century exponentially rose atmospheric carbon dioxide (CO_2) levels. CO_2 occupies a prominent position in anthropogenic greenhouse gases (GHGs) and is widely considered the main contributor to the global temperature rise.

1.1 Global Scenario of carbon dioxide (CO_2) emissions

Gases that trap heat in the atmosphere are called GHGs. The property of CO_2 to emit and absorb thermal radiation makes it a greenhouse gas. Anthropogenic CO_2 enters the atmosphere through burning fossil fuels (coal, natural gas, and oil), solid waste, trees, and other biological materials, and as an outcome of specific chemical reactions on the industrial level. Global carbon emissions from fossil fuels have increased considerably since 1900 (United States Environmental Protection Agency, epa.gov). When scientists first started measuring atmospheric CO_2 consistently in 1958, at the Mauna Lao observatory in Hawaii, the CO_2 level stood at 316 ppm, just a little higher than the pre-industrial level of 280 ppm. In February 2021, it reached an all-time high of 416.75 ppm, almost hitting the 50% increase mark (Fig. 1).

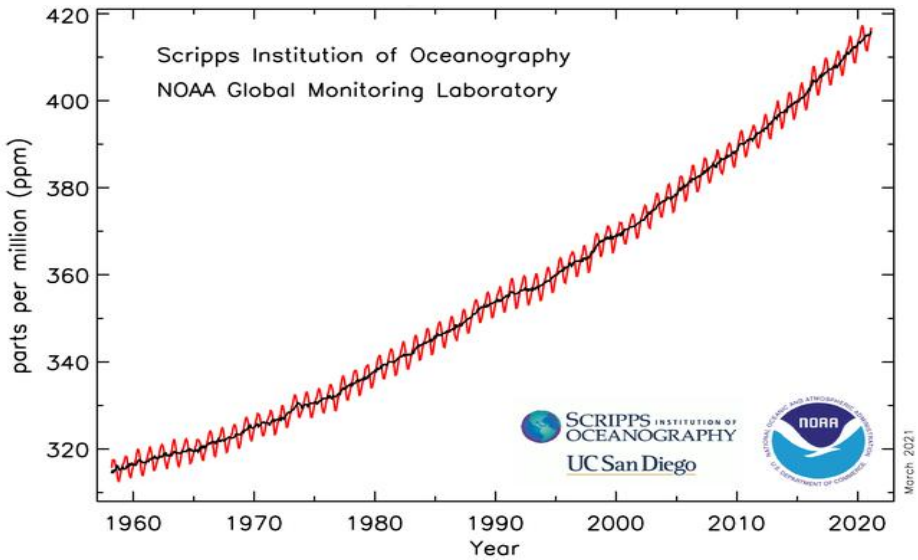

Fig 1. Atmospheric CO_2 at Mauna Loa observatory (esrl.noaa.gov)

The Paris Agreement got enforced in 2016 with the aim of keeping the global temperature rise of this century below 2°C above the pre-industrial level (unfccc.int). Although significant developments have been achieved in renewable energy and energy efficiency to minimize carbon emissions, these are not sufficient to meet the desired target. Additional CO_2 removal from the atmosphere is necessary to bring current atmospheric CO_2 levels under control. Naturally emitted CO_2 are removed from the atmosphere only when plants and microbes use it and oceans absorb it as part of the carbon cycle. However, there is an instantaneous urge to control the linear rise of anthropogenic carbon emissions and develop competent technologies to close the linear carbon chain for sustainable development. The proposition of carbon capture and utilization (CCU) has recently gained great interest as it provides a smart solution. It suggests preventing CO_2 emissions into the atmosphere by carbon capture and converting it into valuable chemical compounds (Cuéllar-Franca & Azapagic, 2015). It allows to close the carbon cycle and address the issue of GHGs emissions thereby reducing carbon footprint.

1.2 Existing and emerging technologies for reducing carbon footprint

Majorly, there are two approaches that are primarily considered and researched for reducing the carbon footprint in the atmosphere (Fig 2), namely, carbon capture and storage (CCS) and carbon capture and utilization (CCU) (Jones et al., 2017).

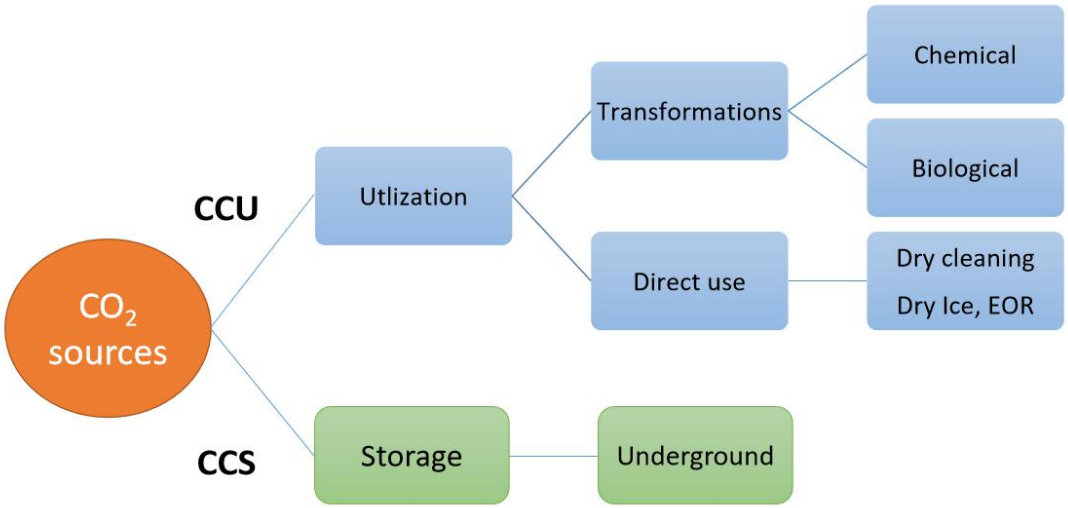

Fig 2. Carbon Capture Storage and Utilization approaches (Adapted from Jones et al., 2017).

In CCS, CO_2 captured from the point sources can be stored underground in geological formations. It reduces carbon emissions into the atmosphere but does not solve the problem completely. In CCU, CO_2 is considered as a feedstock for producing new value-added products. CO_2 can be used directly as a solvent for dry cleaning and enhanced oil recovery (EOR) applications and can be transformed into valuable chemical compounds and fuels by chemical or biological processes (Jones et al., 2017). It can thus promote sustainability and a circular carbon economy.

1.2.1 Carbon capture and storage (CCS)

CCS aims at carbon capture at the point sources and long-term storage. It generally relocates CO_2 from entering into the atmosphere to a much safer site. Here, waste CO_2 is trapped from point sources (e.g. petroleum, cement, steel industries) by various methods represented in fig. 3. It is then liquefied, transported, and stored deep underground in vacant oil and gas reservoirs, deep saline aquifers and oceans beds (Budzianowski, 2017). The high costs associated with gas scrubbing, transport systems, and storing it in porous geological formations at high pressures and temperatures to keep CO_2 in the liquid phase make this strategy very expensive. Moreover, the long-term consequences of stored carbon are still unpredictable (Budzianowski, 2017). In post-combustion capture, CO_2 containing flue gas is collected and stored after combustion takes place. It mostly contains carbon monoxide (CO) and requires further scrubbing to get concentrated CO_2 gas. In Oxy-fuel combustion ample amount of O_2 is supplied during combustion so that complete combustion takes place. Under this condition, the primary products are CO_2 and water, where water can be condensed to get concentrated CO_2 from the exhaust. In pre-combustion capture, the amount of O_2 is carefully controlled so that only a portion of fuel burns. It produces sufficient heat energy to decompose the fuel and produce syngas (H_2, CO_2, CO). Syngas is later processed in water-gas-shift (WGS) to convert CO into CO_2, further increasing CO_2 concentration (Songolzadeh et al., 2014).

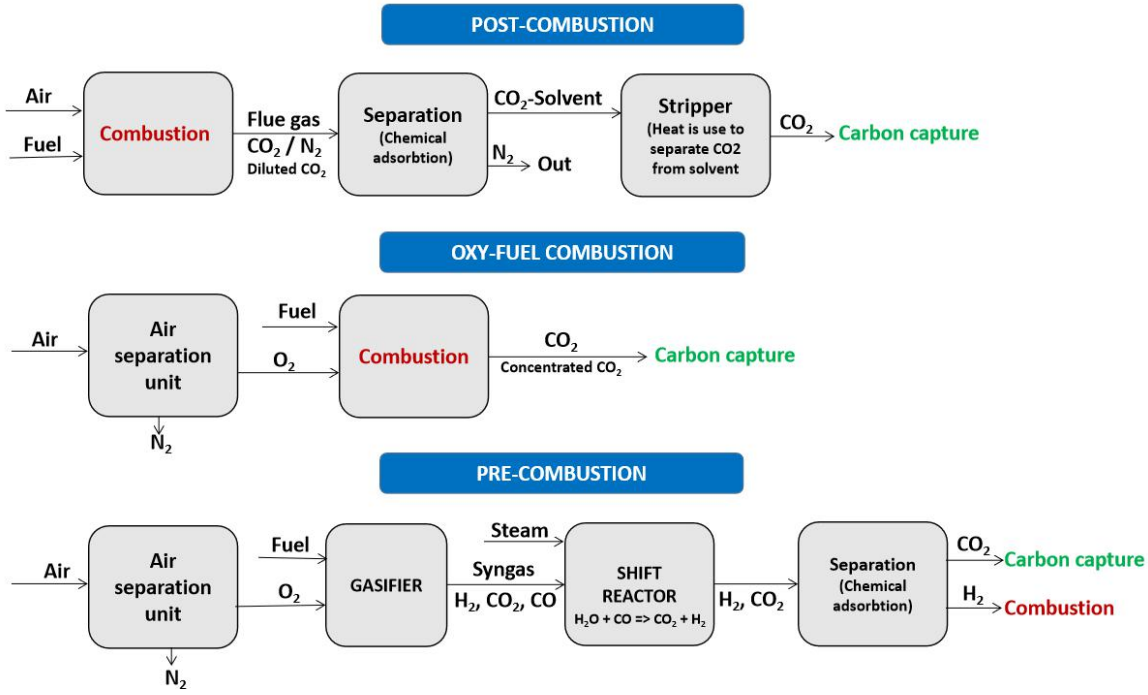

Fig 3: The different methods of carbon capture (Adapted from Songolzadeh et al., 2014).

1.2.2 Carbon capture and utilization (CCU)

CCU aims at carbon capture and its efficient utilization either directly or by converting it into longer carbon chain length products. It is a carbon-neutral technique as it utilizes carbon emitted from the combustion of fossil fuels and thus substitutes their requirement, and therefore controls any additional carbon emissions. It is a reliable long-term solution to close the carbon cycle through various CO_2 utilization approaches. Several biological and chemical methods are proposed and under research and development stages for the utilization of CO_2, as illustrated in fig 4.

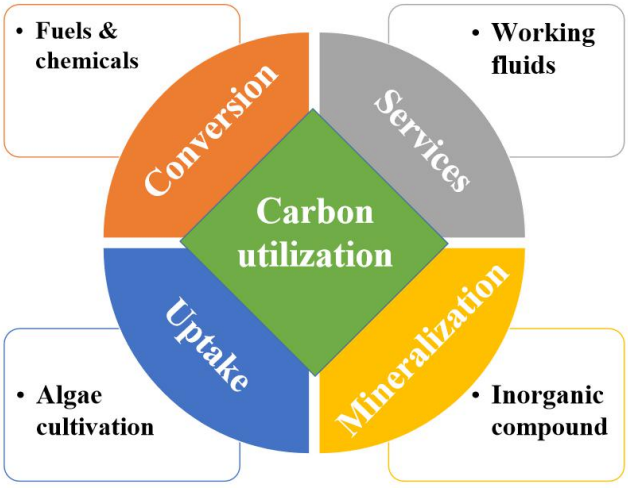

Fig 4. CO_2 utilization pathways (source: netl.doe.gov).

Captured CO_2 can provide various commercial services and products like dry cleaning, dry ice, and enhanced oil recovery application. Mineralization of CO_2 can be done in which CO_2 is reacted with minerals rich in Ca and Mg to form CO2ates (e.g. calcite). CO_2 can be used as a feedstock for algal farming. It can be converted into methanol via chemical reactions like reverse gas phase reactor and Sabatier reaction or electrochemical reduction. Bio-conversion of CO_2 into valuable fuels and chemicals using bio-catalysts is another carbon utilization way (Table 1).

Table 1. Different CO_2 utilization pathways.

Methods	**Applications**	**Products**
Direct Utilization	Direct use of purified CO_2	Enhanced oil recovery (EOR), CO_2 as solvent (Dry cleaning), CO_2 as refrigerant (Dry Ice),
Mineralization	CO_2 reacts with minerals rich in Ca and Mg to form carbonates	Calcite ($CaCO_3$), Magnesite ($MgCO_3$), Dolomite ($CaMg(CO_3)_2$)
Algal Bio-refinery	Algal farming (CO_2 feed to algae)	Wide variety of chemicals and biofuels from algal biomass
Electrochemical conversion	Electrochemical reduction of CO_2	Methane, methanol and formic acid
Chemical conversion	Reverse water gas-shift reaction, Sabatier reaction.	Methane, CO
Bio-conversion	Microbial-mediated conversion of CO_2 via gas fermentation and Bio-electrochemical systems	Organic products, fuels, and fine chemicals

1.3 Bio-electrochemical systems (BES)

Bio-electrochemical systems (BESs) are the emerging biotechnologies that have substantially expanded their scope over the last few years. In BESs, microbes catalyze substrate oxidation using anode as the terminal electron acceptor and substrate reduction using cathode as the electron donor (Bajracharya et al., 2016). Microbial electrosynthesis (MES), a type of BES-based process, relies on microorganisms that can use electrons or energy carriers derived from solid-state electrodes to catalyze the reduction of CO_2 to multi-carbon organic molecules (Rabaey & Rozendal, 2010, Lovley & Nevin, 2013). It also allows converting electrical energy into stable, high energy-density products that can be easily stored, distributed, and consumed as per the demand.

1.3.1 Microbial electrosynthesis (MES)

A MES system consists of an anode, a cathode, and a proton exchange membrane (PEM) separating the two electrode chambers of the reactor (Rabaey & Rozendal, 2010, Bajracharya et al., 2016) (Fig 5). An oxidation reaction occurs in the anode chamber (mostly water oxidation) and produces protons, which diffuse into the cathode chamber via PEM and eventually get reduced to H_2 (at specific potential) by accepting electrons at the cathode. The microbial CO_2 reduction reaction occurs in the cathode chamber. Microbes draw electrons from the cathode either through direct electron uptake or through the utilization of evolved H_2. Here, oxidation of water (H_2O/O_2 ; E'_0=0.82 V *vs.* SHE)(1) is coupled with reduction of H^+ ions (H^+/H_2 ; E'_0= –0.41 V *vs.* SHE)(2), and the resulting cell voltage is negative (–1.23 V), hence external power input is required (Rabaey & Rozendal, 2010).

Anode reaction, $2H_2O$ ------> $4H^+ + O_2 + 4e^-$ (1)

Cathode reaction, $4H^+ + 4e^-$ ----> $4H_2$ (2)

Wood-Ljungdahl pathway, $2CO_2 + 8e^- + 8H^+ \rightarrow 2CH_3COOH$ (3)

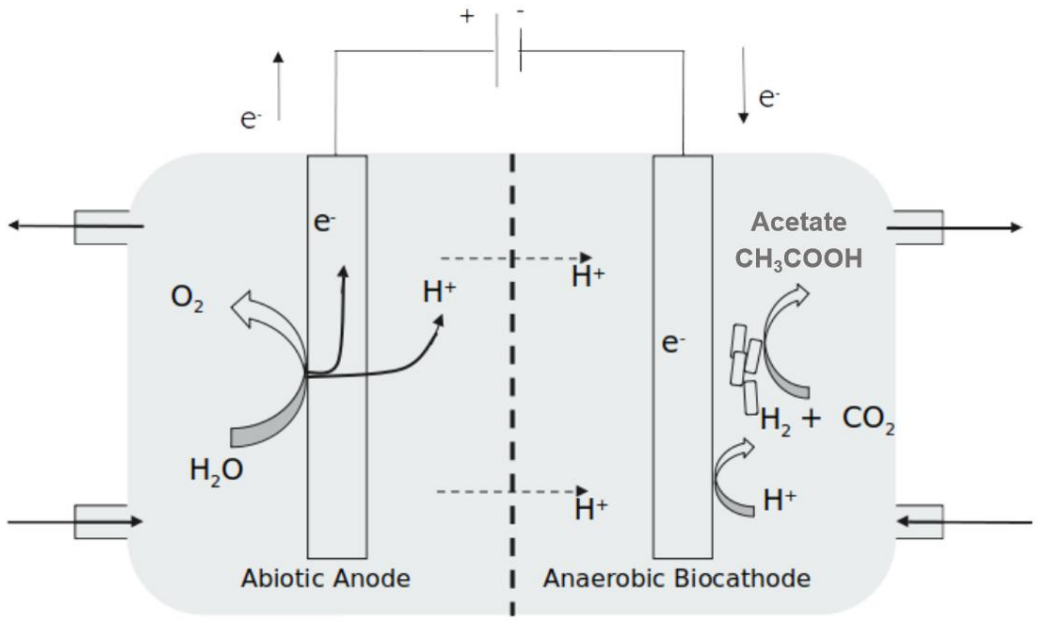

Fig 5. Schematic of a microbial electrosynthesis (MES) system. (Adapted from Bajracharya et al., 2016)

1.3.2 Microbes as the biocatalysts for conversion of CO_2 into acetic acid

A group of microbes known as acetogens use the Wood-Ljungdahl pathway, also known as the reductive acetyl-CoA pathway, to fix CO_2 anaerobically. It was discovered by Ljungdahl (1969). Acetogens use it as their primary mechanism for energy conservation and to synthesize acetyl-CoA and biomass by consuming CO_2 (Lovley, 2011). An acetogen can be a homoacetogen (e.g. *Acetobacterium woodii*) that produces acetate as its only fermentative end product or heteroacetogen (e.g. *Clostridium ljungdahlii),* which produces more than one end products. The Wood–Ljungdahl pathway is relatively simple and works by reducing two molecules of CO_2 in parallel to form acetyl-CoA (Fig 6).

Nevin et al. (2010) reported for the first time that *Sporomusa ovata* could produce acetate from CO_2 at an applied cathodic potential of -0.4 V *vs.* SHE in the MES system. They also reported that *Clostridium ljungdahlii* can produce acetate from CO_2 using electrons from the cathode (Nevin et al., 2010). Enriched mixed microbial cultures dominated by acetogens can also be used for acetate production, as they have shown highly consistent and stable acetic acid production (Patil et al., 2015). Due to the synergistic relationship among different microbial groups, the mixed cultures tend to perform better than pure

cultures in MES. For instance, microaerophiles or facultative microbes present in the mixed culture can scavenge any trace amount of O_2 present in the environment and maintain the anaerobic conditions required for stable bio-catalysis. In the case of pure culture, the product spectrum is narrow, making it easier for down-streaming. In comparison with pure strains (Nevin et al., 2010), mixed cultures (Marshall et al., 2013, Roy et al., 2021) are probably more appropriate for the commercialization of MES due to high biomass growth and acetate production rates besides robustness to environmental disturbances (Marshall et al., 2013). The enriched mixed cultures are dominated mainly by H_2 producing and CO_2 fixing microorganisms (Roy et al., 2021, Ragsdale, 2008).

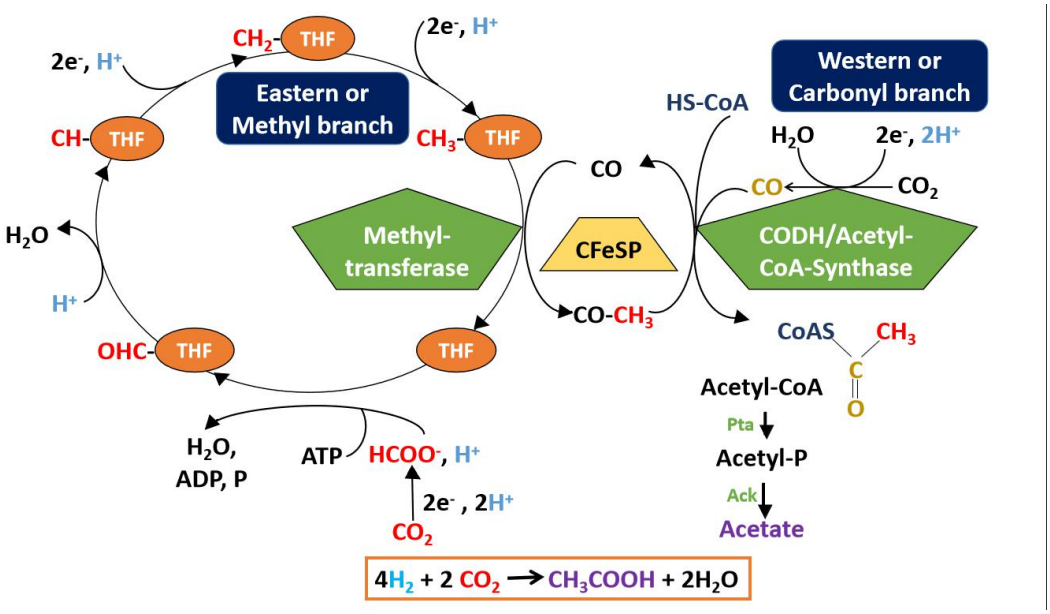

Fig 6. The Wood-Ljungdahl Pathway of CO_2 fixation. (Adapted from agsdale & Pierce, 2008)

1.4 Demand for acetic acid in the global market

The most common end-product of MES with the highest production rate so far is acetic acid. It is an important chemical commodity required to produce several other valuable chemicals (Pal & Nayak, 2017). These chemicals have applications in various industries such as textile, food, automobile, and construction. The growing requirement from these industries is causing the growth in acetic acid demand, which was 12.1 million tons in 2014 and is projected to shoot at 16.8 million tons by 2022 (Caxiano et al., 2020) . The global market for acetic acid is forecasted to reach $11.4 billion by 2024 (IMARC). Fossil fuels-based acetic acid production has a high carbon footprint, and therefore alternative sustainable approaches should be considered (Budsberg et al., 2020). MES, along with some other bio-conversion technologies, have shown promising results. From the 1st demonstration in 2009–10 (Nevin et al., 2010), MES productivity has improved by mani-fold (Prévoteau et al., 2020). It is evident from the fact that the concentration of acetic acid produced via MES has increased from 0.6 g/L to 12.4 g/L in the past decade, and the production rates have also increased from 1.3 $g/m^2/d$, all the way up to 685 $g/m^2/d$ (Prévoteau et al., 2020), (Bian et al., 2020, Flexer & Jourdin, 2019). The investment costs of producing acetate by MES are still relatively high (1.44 £ kg−1, 1770 £ t−1) (Christodoulou & Velasquez-orta, 2016). Therefore, there is need to increase the productivity of acetic acid further and reduce its costs to advance the technology toward the industrial level. However, the existing materials and processes are still insufficient for scaling MES to its practical applications. Bio-cathode materials, selective microbial consortia, and efficient reactor designs are key elements to be addressed. This study mainly focuses on developing a unique tank-type membrane-less single-chamber reactor design for MES. Membrane-less systems can reduce the costs and ohmic losses associated with PEM, but O_2 diffusion towards the cathode needs to be addressed (Butler and Lovley, 2016) since MES activity requires an anaerobic environment.

1.5 Single-chambered membrane-less reactor

In conventional two-chambered MES reactors, membranes add substantial cost, and designing large-scale reactors with two chambers separated by a membrane is challenging (Giddings et al., 2015). The compact nature of a single-chambered membrane-less reactor offers low maintenance, scalability, and ease of handling. Some attempts have been made to upgrade MES reactor design and operating conditions for advancement in MES activity. The possibility of designing reactors without a membrane separating the anode and cathode has also been considered to simplify the MES reactor designs further. For instance, a membrane-less MES reactor was used with CO_2 gas sparged from the bottom of the reactor to mitigate O_2 diffusion from the anode (top) towards the cathode (bottom) by Giddings et al. (2015). However, the acetic acid production rate of 1.7 to 2.2 $g/m^2/d$ and coulombic efficiency of 54 ± 10% was achieved using *Sporomusa ovata,* which was lower than that of 2.7-3.4 $g/m^2/d$ obtained with the same strain using in a two-chambered reactor (Russell, 2013; Zhang et al., 2013). A membrane-less reactor has also been proposed for bio-methane production from CO_2 (Giang et al., 2018). In membrane-less reactors, there is a risk of O_2 produced at the anode to reach the cathode and abiotically consume electrons. It also disturbs the bio-catalytic activity of anaerobic acetogenic microorganisms, which diminishes the reactor efficiency by creating an unfavorable environment for them. Since some knowledge gaps persist in the working of such reactors, it is essential to work on the development of such low maintenance, easy to handle, and scalable reactor designs for the production of acetic acid at higher concentrations at low costs.

2. Objective

✧ To design and test a single-chambered membrane-less MES reactor for acetic acid production with higher coulombic and energetic efficiency.

3. Materials and Methods

3.1 Reactor setup

As shown in the figure 7 and photo 1, a tank-type MES reactor consists of two components made up of borosilicate glass; one gets fixed on top of the other. It is sealed using a rubber ring and a stainless-steel holder, which keeps them together in an airtight manner to be operated in three-electrode configuration mode. The total volume of the reactor is 1500 ml. The total working volume is 1000 ml, out of which 100 ml medium is present in the anode chamber and 900 ml in the cathode chamber. The cathode chamber is pressurized using N_2 gas, which pushes and raises the level of medium in the anode chamber. The sintered sparger is placed below the opening of the anode chamber, which continuously sparges N_2 with minimal flow rate inside the anode chamber, flushing out through the opening at the top along with the O_2 produced by water oxidation reaction at the anode electrode. It is crucial to get rid of O_2 in the anode chamber and restrict it from entering the cathode chamber as the microbial catalysts used are anaerobic, and the presence of O_2 can significantly reduce acetic acid production efficiency.

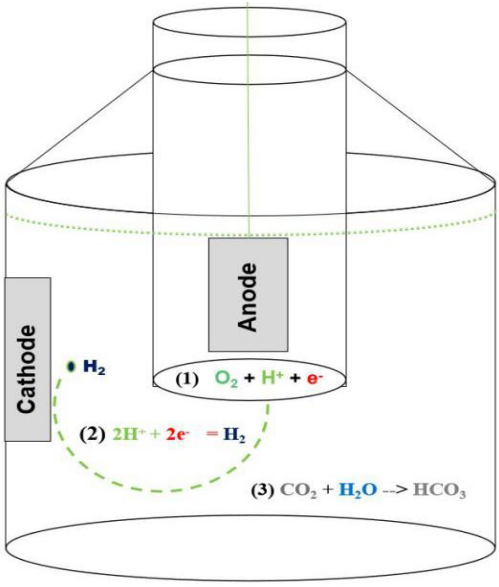

Fig 7. Reactor design and reaction

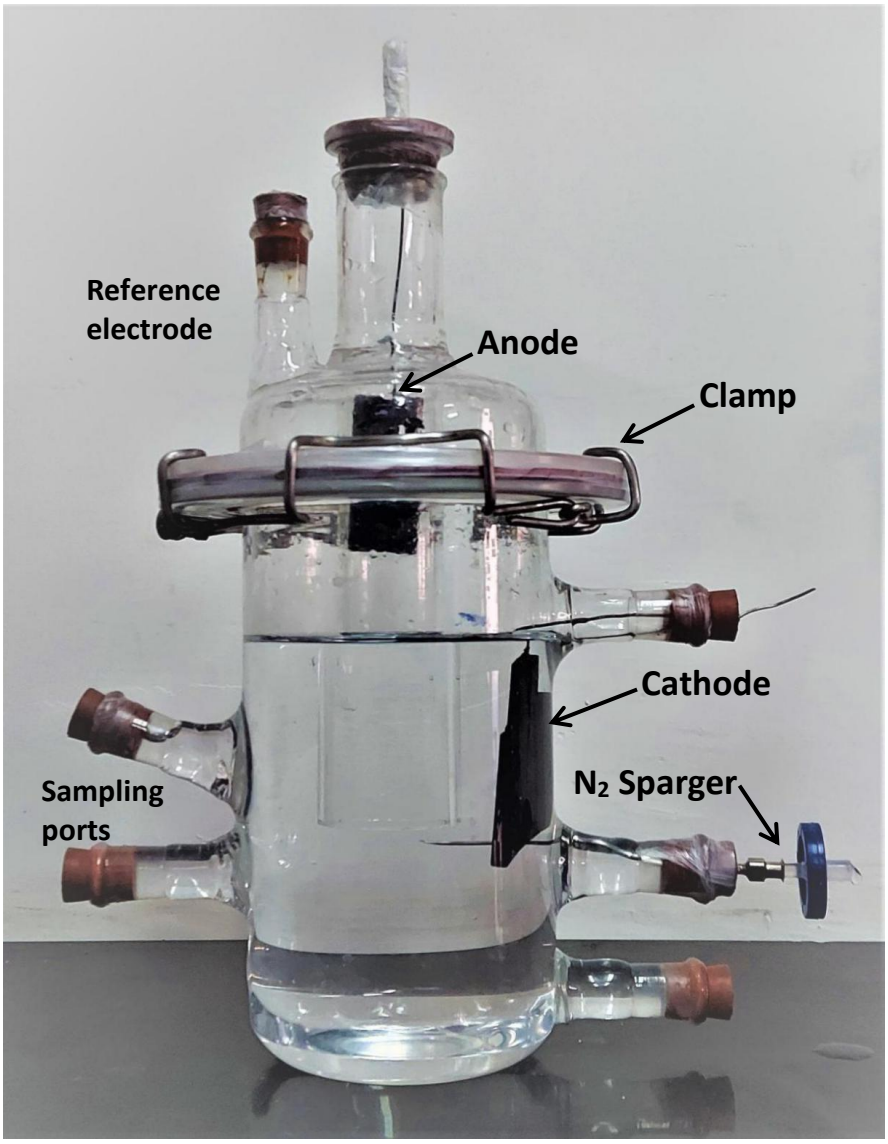

Photo 1: Prototype single-chambered membrane-less reactor.

3.2 Electrode materials, inoculum source and MES experiments

A graphite plate served as a working electrode (i.e. cathode), and a mixed metal oxide coated titanium plate was used as a counter electrode (i.e. anode) initially with 10cm^2 and then 30 cm^2 projected surface area each. The working electrode was pretreated using acid-alkali treatment to remove impurities, and Ag/AgCl (3.5 M KCl, 0.205 V *vs*. SHE) reference electrode was placed near the working electrode. The reactor was filled with the medium prepared as per the composition mentioned in Table 2 (Patil et al., 2015). Bicarbonate (4g/L) was used as the sole carbon source. A magnetic bead with 30 rpm was placed in the reactor to improve mass transfer and prevent microbes from settling down.

According to the abiotic cathode cyclic voltammetry data, a cathodic potential of -1.2 V (*vs.* Ag/AgCl) was applied and maintained using Potentiostat (VMP3, Bio-Logic Science Instruments, France) to ensure a continuous supply of H_2 to the microorganisms. The MES experiments were conducted in two batch cycles. Activation polarization was performed at -0.6 V before inoculation to stabilize the reactor and bring the electrodes at equilibrium electrochemically. The spent medium was replenished at the end of each batch cycle.

An enriched mixed microbial culture from anaerobic sludge at a municipal wastewater treatment plant (Mohali, India) dominated by acetogens was used as the inoculum source. During enrichment, 2- bromoethanesulfonate was used as the inhibitor for methanogens, while CO_2 and H_2 were the sole sources of carbon and energy, respectively (Roy et al., 2021). All microbial and bioelectrochemical experiments were conducted at a controlled temperature of 28 ± 2 °C, and the electrode potential data are reported against Ag/AgCl (3.5 M KCl) reference electrode (0.205 V *vs.* SHE), if not stated otherwise. Based on the studies of different forms of dissolved CO_2 (Fig 8) (Taylor et al., 2012), the pH of working medium was maintained at 7 to improve the solubility of CO_2 gas into bicarbonate form.

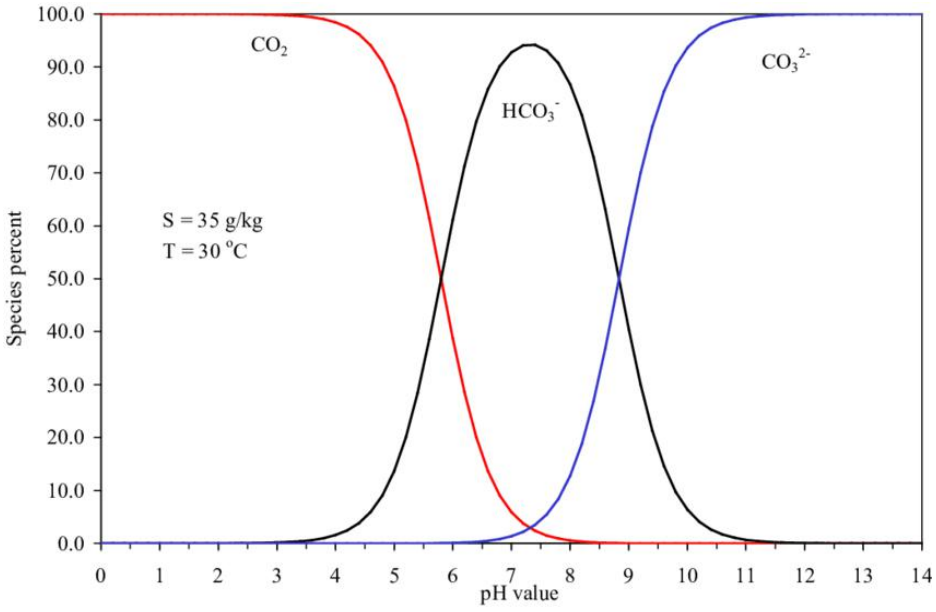

Fig 8. Speciation diagram of CO_2 , HCO_3 and CO_3^{2-} as a function of pH value at 30°C (Source: Taylor et al. 2012).

Table 2. Composition of the growth cum electrolyte medium

Component	Quantity	Component	Quantity
$K_2H_2HPO_4$	5.35 g/L	Trace metal solution (Table S-1)	1 mL/L
KPO_4	2.62 g/L	Vitamin solution (Table S-2)	2.5 mL/L
NH_4Cl	0.25 g/L	Selenium – tungstate solution (Table S-3)	1 mL/L
KCl	0.5 g/L	2-bromoethanesulfonate	6.4 g/L
$CaCl_2 \cdot 2H_2O$	0.15 g/L	$Na_2S \cdot 9H_2O$	0.3 g/L
$MgCl_2 \cdot 2H_2O$	0.60 g/L	Resazurin	0.5 g/L (0.1% stock solution)

3.3 Major experiments

3.3.1 Control experiments

Two control experiments, namely biotic unconnected (inoculated but not connected electrochemically, thus no electron source), and abiotic connected (electrochemically connected but uninoculated, thus only abiotic or electrochemical catalysis) were conducted to confirm the microbially catalyzed electricity-driven bio-production process.

3.3.2 Preliminary MES experiments with the reactor

Electrode surface area to volume ratio was kept at 10 cm^2 per litre of medium. N_2 sparging flow rate in the anode chamber was set at 25ml/min, and the cathode was placed 3 cm below the headspace of the reactor.

3.3.3 Improvisation of the reactor's conditions

Electrode surface area to volume ratio was improved to 30cm^2 per litre of medium. Also the N_2 sparging in the anode chamber was increased to 60ml/min, and the cathode was placed 7cm below the headspace of the reactor to increase the retention time of evolved H_2.

3.4 Sampling and Analysis

Daily 2ml of the bulk phase and gas samples were taken from the cathode chamber. During experimentation, different parameters were analyzed, namely pH, optical density, organic acids, gas composition, and reduction current (Table 3). pH and growth were monitored at regular intervals. Potentiostat (VMP3, Bio-Logic Science Instruments, France) was used to maintain the constant working potential of -1.2V *vs.* Ag/AgCl at the working electrode (cathode). Cyclic voltammetry at different conditions, namely blank, after inoculation and bio-cathode was performed in the range -1.4 to -0.2V at a scan rate of 1mV/s. Organic acids were analyzed using HPLC-RID (Agilent 1260 Infinity II, RID Detector, Hiplex H column, 5µM H_2SO_4 mobile phase, flow rate 0.5mL/min, temperature 50◦C). The head-space gas composition was analyzed using GC-TCD (Agilent 490 Micro GC). It was equipped with three channels for different gases [Channel 1: Column- Molecular sieve for H_2, carrier gas- Ar; Channel 2: Column- Molecular sieve for O_2, N_2, CO and CH_4, carrier gas– He; Channel 3: Column- Pora plot U for CO_2 and H_2S, carrier gas– He].

Microscopy was done to observe microbial cells in the bulk sample of the reactor. Gram staining was performed on the microbial samples before observing under a bright-field microscope.

Table 3. Sampling and analysis of different operational parameters

Analysis	Instrument
C1-C4 Organics (acids and alcohols) production	HPLC (Agilent-1260 Infnity II)
Microbial growth as OD_{600}	UV–VIS Spectrophotometer (Photo-lab 7600)
pH	pH meter (Oakton PC2700)
Gas samples (H_2, CO_2, O_2, CH_4 and H_2S)	GC-TCD (Agilent-490 Micro GC)

3.5 Calculations

The acetic acid production and electrochemical data for two batch cycles were considered to check the product titer, production rates and coulombic efficiency of the reactor. They are calculated based on information available elsewhere (Patil et al., 2015).

3.5.1 Coulombic efficiency

Current is drawn from the system by the microbes for MES of acetic acid. Coulombic efficiency (ε_C) is equal to the percentage of electrons that is recovered (electron recovery) in the target product. It is calculated according to the following formula.

$$\varepsilon_C = \frac{F\ M_p\ \Delta e}{\int i\ dt}$$

where

F : Faraday's constant (96485 C mol^{-1})

M_p : Moles of produced product

$\int i\ dt$: Integration of current supplied over the specific time period. (total charge drawn over certain period of time to produce the product)

Δe : Number of electrons required for the production of one mole of a particular target product

4. Results and discussion

Microorganisms require electrons to generate energy. In MES, they can either use electrons via direct electron transfer (DET) from the cathode or by indirect electron transfer using an H_2 energy carrier. In both control experiments, namely abiotic connected and biotic unconnected, neither acetic acid production nor microbial growth was observed.

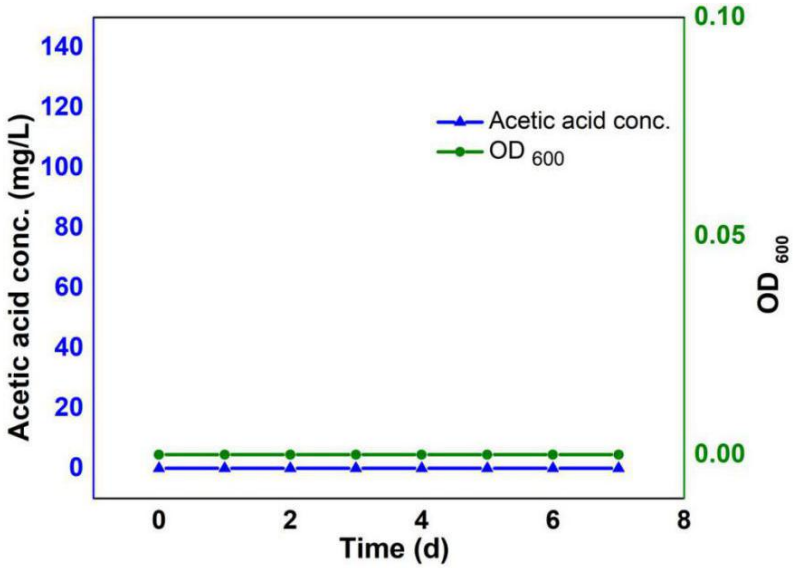

Fig 9. Acetic acid production profile for the abiotic control reactor

In the abiotic control experiment (Fig 9), electrochemical reduction of bicarbonate to acetic acid did not occur at the cathode potential of -1.2 V. Also, a low and stable reduction current production was observed (Fig. 10).

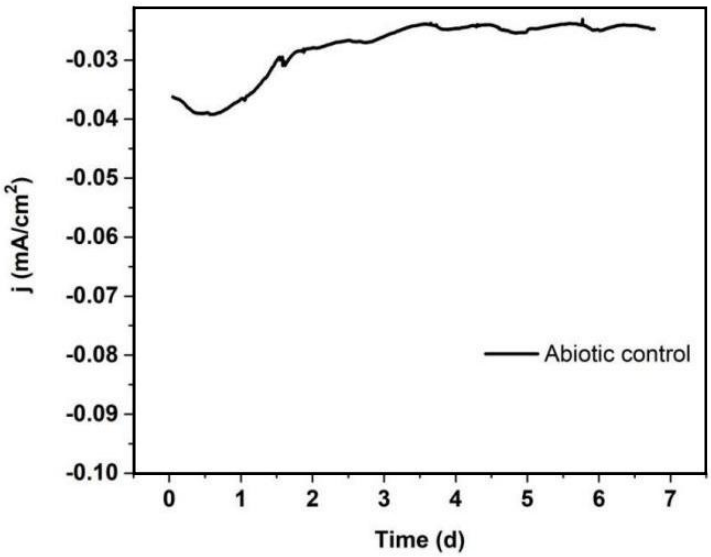

Fig 10. Chronoamperogram of the abiotic control experiment ($E_{cathode}$ = -1.2 V).

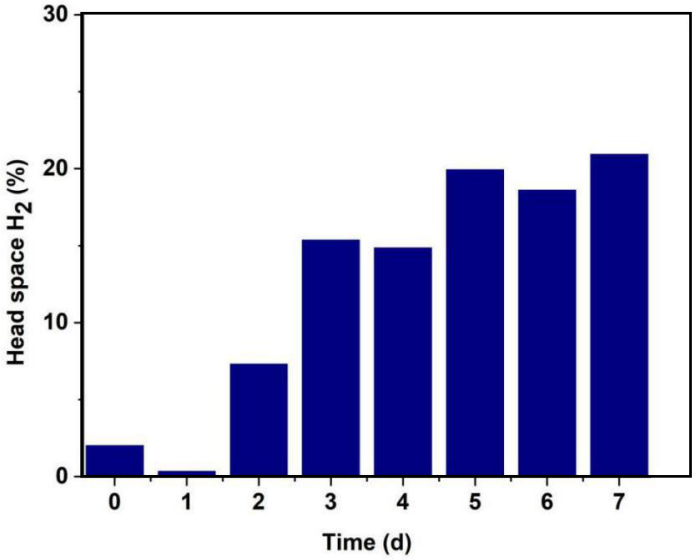

Fig 11. Head-space H_2 gas data of the abiotic control experiment.

Since microbes were absent in this condition, continuous accumulation of electrochemically produced H_2 was observed (Fig 11).

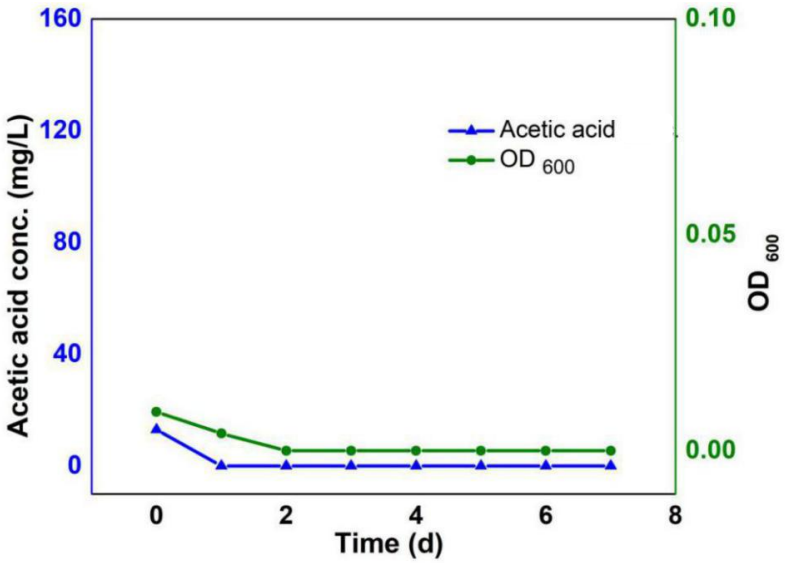

Fig 12. Acetic acid production and growth profiles of the biotic control experiment.

In the biotic control unconnected experiment (Fig 12), due to the absence of an energy source, there was no production of acetic acid and the growth of microbes. Initial acetic acid and OD present on days 1 and 2 were because of the residual amount in the inoculum source. In an open circuit (unconnected) condition, no electrochemical H_2 was observed. It confirms that no hydrogen or any other energy source production occurred without a closed circuit (connected) applied potential condition in these systems.

4.1 Preliminary MES experiments with the reactor

A) Production and growth profile data

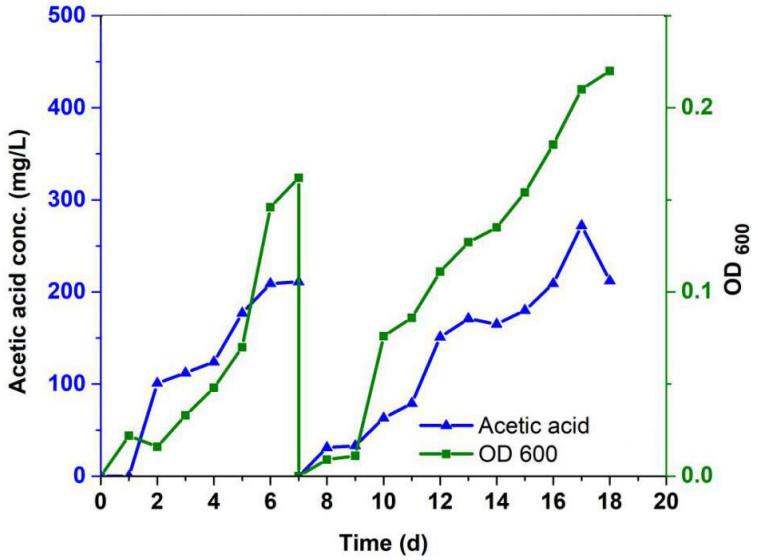

Fig 13. Production and growth profile of the MES experiment

Two batch cycles were run to check the production of acetic acid. A maximum titer achieved was 272 mg/L with volumetric and cathode surface area-based production rates of 0.02 g/L/d and 27.2. g/m^2/d, respectively. The increase in OD was linked to the increase in acetic acid concentration (Fig. 13).

B) Electrochemical analysis

In cyclic voltammetry analysis (Fig 14), at biocathode (i.e. cathode with microbes), a decrease in the H_2 evolution over-potential was observed (from -1 V in blank CV to -0.85 V *vs.* Ag/AgCl with biocathode). The shift in over-potential can be attributed to the presence of microbes at the cathode or in its vicinity. This phenomenon of a shift in over-potential confirms the bio-catalytic activity of microbes in the tank-type reactor. Also, the reduction current was more at the biocathode than the abiotic cathode (blank).

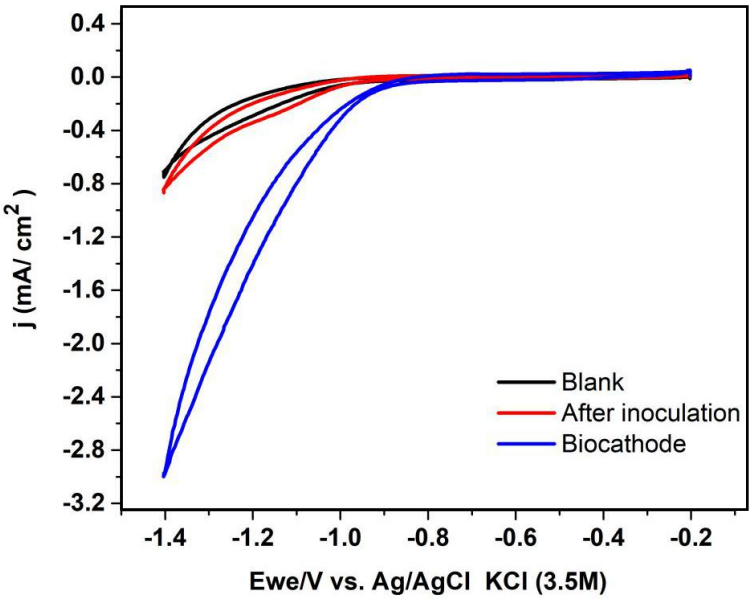

Fig 14. Comparison of cyclic voltammograms recorded at different conditions in preliminary MES experiments.

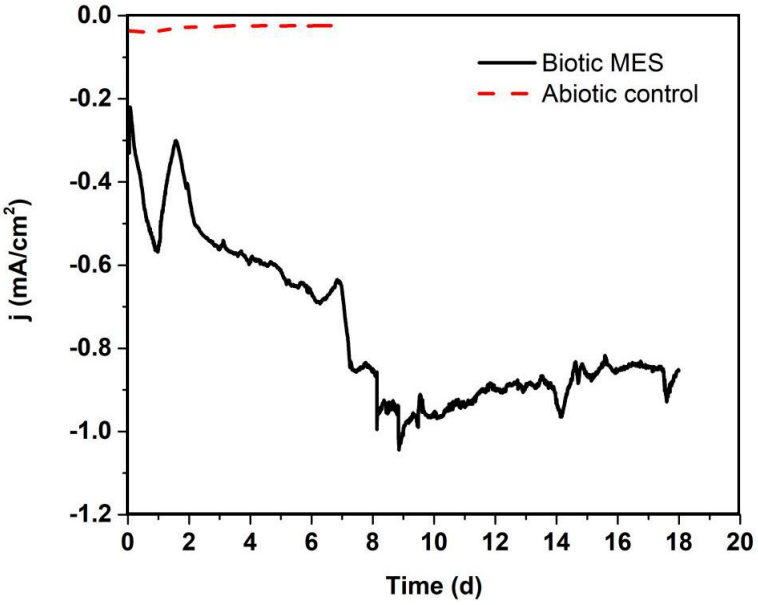

Fig 15. Comparison of chronoamperograms of the abiotic control and biotic MES experiments (E_{cat} = -1.2V vs Ag/AgCl)

From the chronoamperograms comparison data between abiotic and biotic experiments (Fig 15), it is clear that the presence of microbes caused the increase in reduction current. Microbes present in the medium utilize electrons to perform bioelectrocatalytic activity. It took two days for microbes to acclimatize to the new environment, and then they started performing bio-catalytic activities. Chronoamperometry data correlates with the acetic acid production and growth profile data of the experiment (Fig 13). The coulombic efficiency in acetic acid was 14 % in the preliminary MES experiment.

4.2 Improvisation of the reactor's conditions

Based on the low acetic acid concentration and coulombic efficiency data of the preliminary experiments, further optimization of the reactor components was done. It was observed during preliminary experiments that electrode surface area to medium volume was poor (10cm^2 per liter). Also, a trace amount of O_2 was present in the cathode chamber based on the GC analysis of headspace gas composition. Hence, experiments with larger electrodes (30cm^2 each) and at an increased N_2 sparging flow rate of 60ml/min compared to 25ml/min in the preliminary experiment were conducted.

A) Production and growth profile analysis

Both growth and acetic acid production increased after the lag phase of two days and leveled off after the 8th day (stationary phase) in cycle 1. A similar trend was observed in cycle 2, but both production and growth improved further. The growth profile correlates well with the production data in both cycles (Fig. 16). In the improvised reactor experiments, the maximum acetic acid titer achieved was 0.6 ± 0.4 g/L (Fig 16), and the volumetric and cathode surface area-based production rates were 0.06 ± 0.04 g/L/d and 20.0 ± 14. g/m^2/d, respectively.

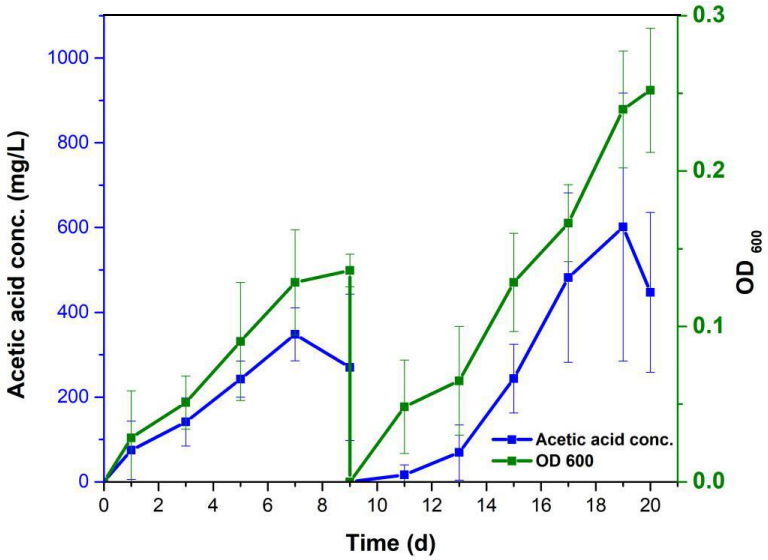

Fig 16. Growth and acetic acid production profiles (n=3) of the MES experiments conducted at improved reactor conditions.

B) Electrochemical analysis

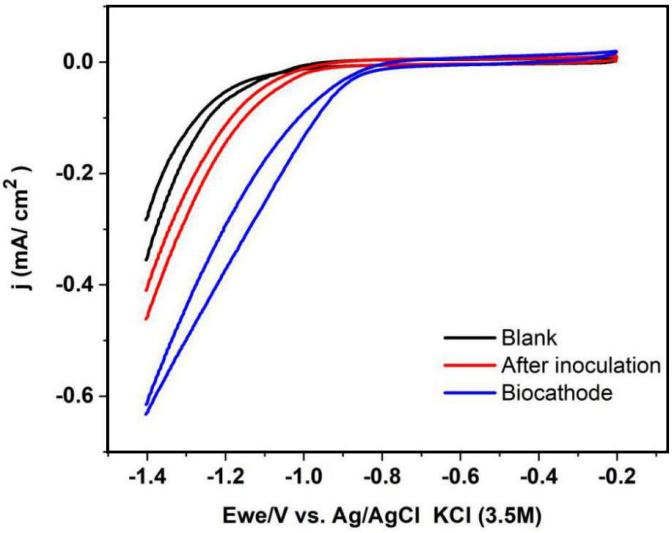

Fig 17. Comparison of cyclic voltammograms at different conditions in the MES experiments conducted with improved reactor conditions. (scan rate: 1 mV/s)

In cyclic voltammograms (CVs) (Fig. 17), no redox peaks at the abiotic cathode (blank) indicate the absence of any redox-active moieties at the cathode surface or in the catholyte of the reactors in the uninoculated condition. By comparing blank CV (abiotic) with biocathode CV (biotic), it can be seen that the H_2 evolution potential of biocathode (i.e. -0.8 V) is higher than the blank CV (i.e. -1.0 V). This shift in H_2 evolution potential by ~0.2 V suggests lowering of the H_2 evolution over-potential at the biocathode due to the presence of microbes. Moreover, the cathodic current drawn at any cathode potential is higher in the case of biocathode than the abiotic cathode due to the presence of microbial catalysts (e.g. at -1.4 V, the current density of biocathode is -0.6 mA/cm^2 and blank is -0.4 mA/cm^2), suggesting enhancement in bioelectrocatalysis in the MES system. The improved electrocatalytic activity and reduced H_2 evolution over-potential can be attributed to microbial activity at the cathode of the MES system.

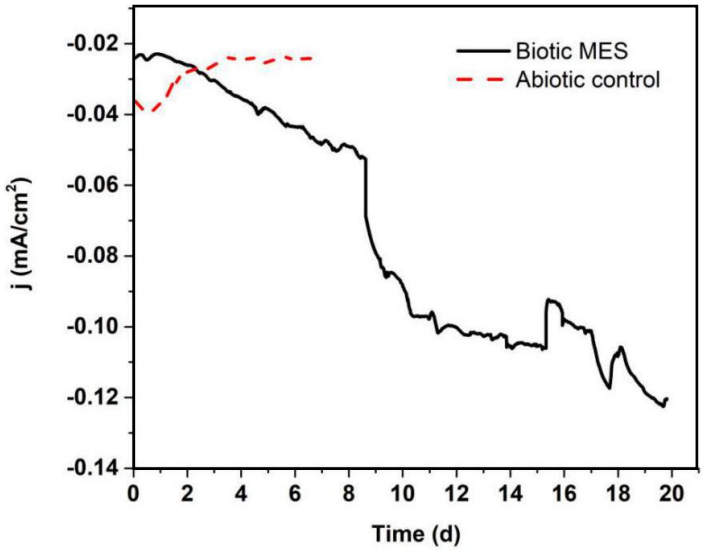

Fig 18. Comparison of chronoamperograms for the abiotic control and biotic MES experiments conducted at improved reactor conditions. ($E_{cathode}$: -1.2 V *vs.* Ag/AgCl)

In chronoamperometry (CA) analysis (Fig. 18), during biotic activity at a constant potential of -1.2 V, there was a significant rise in current drawn from the system for subsequent days as compared to the abiotic control. It can be attributed to the enzymatic activity of microbes present at the cathode surface or in the working medium. More current was drawn from the system due to the presence of bio-catalysts. There was a gradual rise in reduction current from day one itself, which correlates with the growth and acetic acid production profiles (Fig. 16). As there was no significant increase in reduction current observed in abiotic control, it can be confirmed that the increased current in biotic experiments was due to the microbes present on the cathode and in the medium.

C) Headspace gas analysis

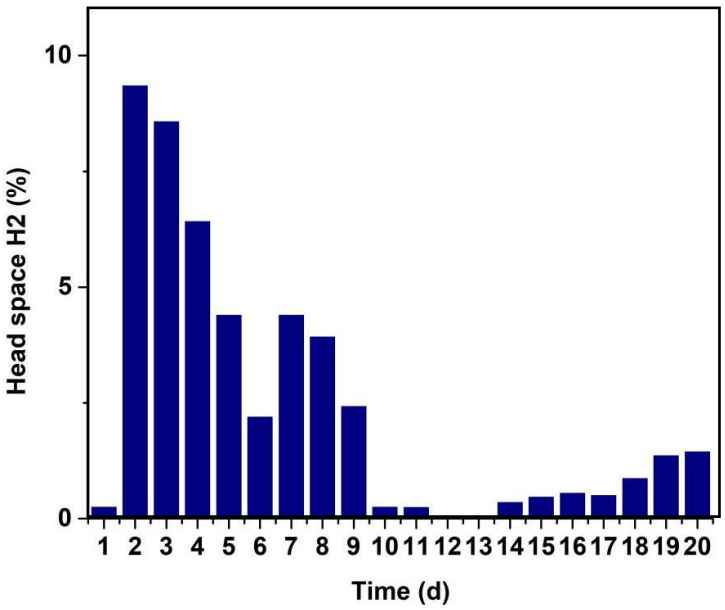

Fig 19. Headspace H_2 gas data of the MES experiments conducted with improvised reactor conditions.

From GC data of the MES experiment of improvised setup (Fig 19), it can be seen that the electrochemically produced H_2 present in the head-space of the reactor decreased over the batch cycle. During the lag period, microbes take time acclimatizing to the new environment. Hence initially, less H_2 is consumed, and gradually, most H_2 in the head-space is consumed by microbes. At the end of the cycle, H_2 started to get accumulated (after 8^{th} day) since the microbes entered the stationary growth phase.

D) Microbial analysis

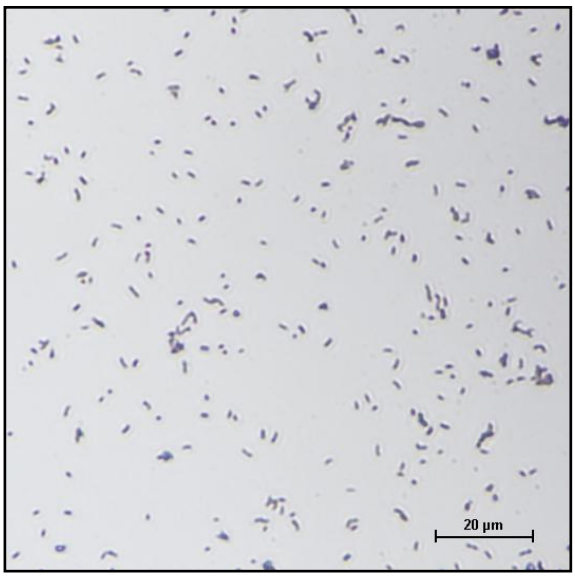

Fig 20. Bright-field microscopic image of the enriched mixed culture inoculated in the reactor.

Enriched mixed culture is dominated by acetogens, which are bacilli (rod) and cocci (round in shape (fig 20). It confirms the presence of microbes in the bulk phase of the reactor. Further scanning electron microscopy of the electrode and community analysis of the culture can provide additional insights into the microbial consortia involved in the MES process.

4.3 Data interpretation

Results from preliminary experiments demonstrated a proof-of-concept of the single-chambered membrane-less reactor. With a production rate of 0.02 g/L/d, the coulombic efficiency of the experimental setup was 14 %. The efficiency of the reactor can be improved by optimizing several operational parameters. Few parameters and conditions were considered to improve the efficiency of the reactor, as mentioned in section 2.5.3. The production rate improved to 0.06 ± 0.04 g/L/d and coulombic efficiency to 85 ± 34% with improvised conditions. Higher production titer and rate compared to the preliminary experiment are due to a larger electrode surface area (as well as better electrode:electrolyte ratio), improved hydrogen retention time by increasing the distance between headspace and cathode, and improved sparging condition to restrict O_2 from entering the cathode chamber. A high percentage recovery of electrons in acetic acid suggests minimum electron losses in other unwanted products or processes.

5. Conclusions

The sole purpose of this study was to design and investigate the performance of a single-chambered membrane-less MES reactor. It reduces ohmic losses caused due to the presence of PEM and distance between the electrodes that are hallmarks of the typical two-chamber H-type reactors. The tested design makes it a simple, compact, and scalable system with higher coulombic efficiency. The proof-of-concept membrane-less system with unique cathode and anode placements was successfully demonstrated for MES of acetic acid from CO_2. The maximum acetic acid titer of 0.6 ± 0.4 g/L with volumetric and cathode surface area-based production rates of 0.06 ± 0.04 g/L/d and 20.0 ± 14. g/m^2/d, respectively, was achieved with this reactor. Coulombic efficiency of 85 ± 34 % in acetic acid was achieved. The high coulombic efficiency of this reactor can be attributed to limited electron losses in other products. Bioproduction at low E_{cell} of -2.6 V can be attributed to the absence of PEM and shorter distance between the electrodes in the reactor.

Further optimization work is required to maximize acetic acid production rate by increasing the cathode surface area, increasing inter-phase between headspace and medium for better dissolution of CO_2 and H_2, and by modifying anode chamber design for efficiently restricting O_2 from escaping into the cathode chamber at a minimal N_2 flow rate.

6. Upgradation of the reactor

A proof-of-concept of the proposed tank-type reactor was demonstrated with a prototype design. For further advancement, we considered the following parameters.

I. Electrode surface area to volume ratio

As confirmed from the MES experiments, improving electrode surface area from 10 cm^2 to 30 cm^2 enhanced the maximum production titer and production rates. Hence, in the upgraded tank-type reactor (Figs. 21, 22), cathode and anode of up to 140 cm^2 projected surface each can be used. It will improve the electrode surface area to volume ratio of the reactor to 90 cm^2/L.

II. Headsapce gas dissolution rate and solubility

The placement of a larger cathode at the base also increases the inter-phase between headspace and medium, which may improve the dissolution rate of the gases. In the upgraded tank-type reactor, the cathode chamber can be pressurized by sparging excess gas. It will raise the medium level in the anode chamber. It will not only improve the solubility of gases in the cathode chamber but also provide extra space for a larger anode to be placed in a narrow anode chamber. Since the cathode chamber is already pressurized and the anode chamber is not, the O_2 produced in the anode chamber will not readily dissolve in the catholyte. The chances of any other gas dissolving into the anode chamber are minimal.

III. Presence of O_2 in the headspace and N_2 sparging flow rate

By narrowing the anode chamber opening inside the reactor from 7 cm^2 (D = 3) to 3 cm^2 (D =2) (Fig. 21), chances of O_2 escaping into the reactor from the anode opening will be minimized. In this case, O_2 can be restricted into the anode chamber even by minimal N_2 sparging flow rate, improving the overall efficiency of the whole process.

IV. Ohmic losses caused by the distance between electrodes

The distance between electrodes can be minimized from 9 cm to 4 cm in the upgraded reactor (Fig. 21), reducing the ohmic losses of the reactor. Narrowing anode opening can also affect H^+ flow from anode to cathode; however, increasing the mixing rate using magnetic bid can improve the flow of H^+ into the cathode chamber.

V. Retention time of evolved H_2

It was observed that placing a cathode near the base can improve acetic acid production as retention time for H_2 increases due to the cathode placement (Section 3.3). However, placing a larger cathode at the base of the existing reactor is not possible, and in the existing reactor, there are more chances of evolved H_2 to escape from the anode opening itself considering its bigger opening. Hence, in the upgraded tank-type reactor, the cathode can be placed at the base of the reactor, which would improve the retention time of H_2.

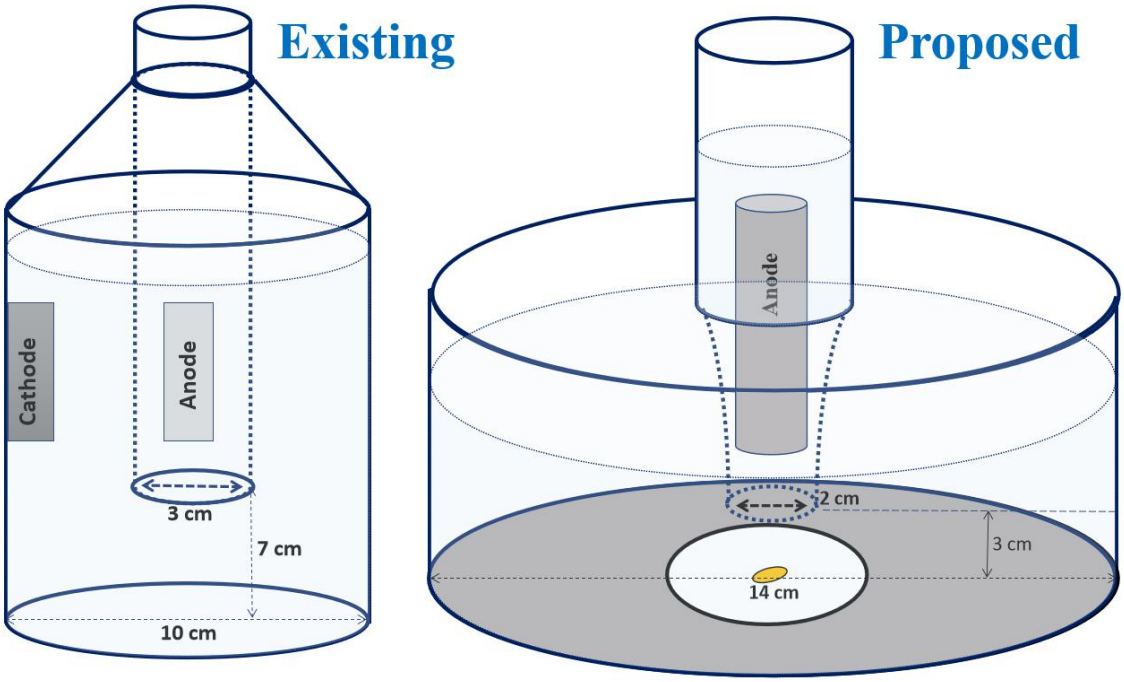

Fig 21. Comparison of the existing and upgraded reactors.

The proposed reactor is compact and have the scale-up potential up to 2L (Fig 22). Some parameters that can be investigated with the improved reactor include direct CO_2 feed, improved cathode materials, and testing with pure cultures.

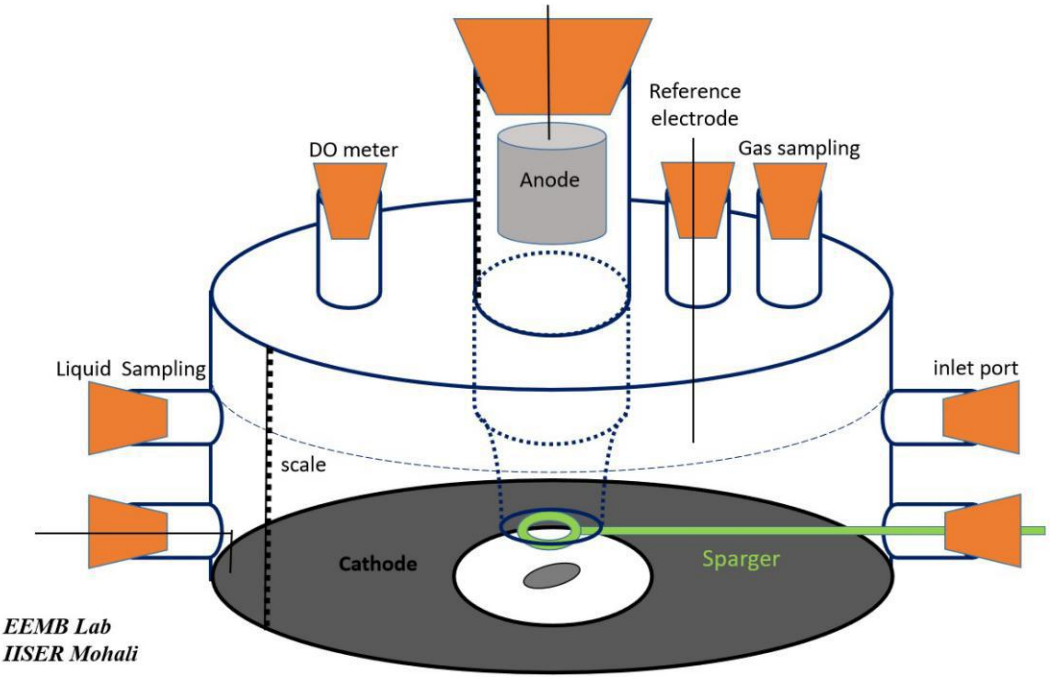

Fig 22. Schematic of the proposed reactor with electrode placement and ports.

To summarize, MES without a membrane separating the anode and cathode reactions can primarily simplify the future reactor designs and lower the construction costs/CAPEX. It can serve as a starting point for developing industrially relevant reactor designs and may eventually contribute to the transitioning of MES technology from lab-scale to industrial level.

✧ Bibliography

Bajracharya, S., Sharma, M., Mohanakrishna, G., Dominguez, X., Strik, D. P. B. T. B., & Sarma, P. M. (2016). An overview on emerging bioelectrochemical systems (BESs): Technology for sustainable electricity , waste remediation , resource recovery , chemical production and beyond. Renewable Energy. https://doi.org/10.1016/j.renene.2016.03.002

Banerjee, A., Leang, C., Ueki, T., Nevin, K. P., & Lovley, D. R. (2014). Lactose-Inducible System for Metabolic Engineering of Clostridium ljungdahlii. 80(8), 2410–2416. https://doi.org/10.1128/AEM.03666-13

Bian, B., Bajracharya, S., Xu, J., Pant, D., & Saikaly, P. E. (2020). Bioresource Technology Microbial electrosynthesis from CO 2 : Challenges , opportunities and perspectives in the context of circular bioeconomy. Bioresource Technology, 302(January), 122863. https://doi.org/10.1016/j.biortech.2020.122863

Budsberg, E., Vera, R. M., Crawford, J. T., Bura, R., & Gustafson, R. (2020). Biotechnology for Biofuels Production routes to bio - acetic acid : life cycle assessment. Biotechnology for Biofuels, 1–15. https://doi.org/10.1186/s13068-020-01784-y

Budzianowski, W. M. (2017). Implementing carbon capture , utilisation and storage in the circular economy. 12(2), 272–296.

Butler, C. S., & Lovley, D. R. (2016). How to sustainably feed a microbe: Strategies for biological production of carbon-based commodities with renewable electricity. Frontiers in Microbiology, 7(NOV), 1–6. https://doi.org/10.3389/fmicb.2016.01879

Caxiano, I. N., Junqueira, P. G., Mangili, P. V., & Prata, D. M. (2020). Eco-efficiency analysis and intensification of the acetic acid purification process. Chemical Engineering and Processing - Process Intensification, 147(November), 107784. https://doi.org/10.1016/j.cep.2019.107784

Christodoulou, X., & Velasquez-orta, S. B. (2016). Microbial electrosynthesis and fermentation : An economic evaluation for acetic acid production from CO 2 and CO. https://doi.org/10.1021/acs.est.6b02101

Cuéllar-Franca, R. M., & Azapagic, A. (2015). Carbon capture, storage and utilisation technologies: A critical analysis and comparison of their life cycle environmental impacts. Journal of CO2 Utilization, 9, 82–102. https://doi.org/10.1016/j.jcou.2014.12.001

Flexer, V., & Jourdin, L. (2019). Purposely Designed Hierarchical Porous Electrodes for High Rate Microbial Electrosynthesis of Acetate from Carbon Dioxide. https://doi.org/10.1021/acs.accounts.9b00523

Franks, A. E. (2015). Microbial electron transport and energy conservation – the foundation for optimizing bioelectrochemical systems. 6(June), 1–18. https://doi.org/10.3389/fmicb.2015.00575

Giang, H., Zhang, J., Zhu, Z., Suni, I. I., & Liang, Y. (2018). Single-chamber microbial electrochemical cell for CH4 production from CO2 utilizing a microbial consortium. International Journal of Energy Research, 42(3), 1308–1315. https://doi.org/10.1002/er.3931

Giddings, C. G. S., Nevin, K. P., Woodward, T., Lovley, D. R., & Chen, L. (2015). Simplifying microbial electrosynthesis reactor design. 6(May), 1–6. https://doi.org/10.3389/fmicb.2015.00468

Jones, C. R., Olfe-kräutlein, B., Naims, H., Armstrong, K., & Jones, C. R. (2017). The Social Acceptance of Carbon Dioxide Utilisation : A Review and Research Agenda. 5(June), 1–13. https://doi.org/10.3389/fenrg.2017.00011

Ljungdahl, L. G. (1969). Total synthesis of acetate from CO2 by heterotrophic bacteria. Annual Review of Microbiology, 23(2), 515–538. https://doi.org/10.1146/annurev.mi.23.100169.002503

Lovley, D. R. (2011). Minireview Powering microbes with electricity : direct electron. 3, 27–35. https://doi.org/10.1111/j.1758-2229.2010.00211.x

Lovley, D. R., & Nevin, K. P. (2013). Electrobiocommodities : powering microbial production of fuels and commodity chemicals from carbon dioxide with electricity. Current Opinion in Biotechnology, 24(3), 385–390. https://doi.org/10.1016/j.copbio.2013.02.012

Marshall, C. W., Ross, D. E., Fichot, E. B., Norman, R. S., & May, H. D. (2013). Long-term Operation of Microbial Electrosynthesis Systems Improves Acetate Production by Autotrophic Microbiomes.

Nevin, K. P., Woodard, T. L., & Franks, A. E. (2010). Microbial Electrosynthesis : Feeding Microbial Electrosynthesis : Feeding Microbes Electricity To Convert Carbon Dioxide and Water to Multicarbon Extracellular Organic. https://doi.org/10.1128/mBio.00103-10.Editor

Nie, H., Zhang, T., Cui, M., Lu, H., Lovley, D. R., & Russell, T. P. (2013). Improved cathode for high efficient microbial-catalyzed reduction in microbial

electrosynthesis cells. Physical Chemistry Chemical Physics, 15(34), 14290–14294. https://doi.org/10.1039/c3cp52697f

Pal, P., & Nayak, J. (2017). Acetic Acid Production and Purification : Critical Review Towards Process Intensification Acetic Acid Production and Puri fi cation : Critical Review Towards Process Intensi fi cation. 2119. https://doi.org/10.1080/15422119.2016.1185017

Patil, S. A., Arends, J., Vanwonterghem, I., Van, J., Guo, K., Tyson, G., & Rabaey, K. (2015a). Selective enrichment establishes a stable performing community for microbial electrosynthesis of acetate from CO2. https://doi.org/10.1021/es506149d

Patil, S. A., Gildemyn, S., Pant, D., Zengler, K., Logan, B. E., & Rabaey, K. (2015b). A logical data representation framework for electricity-driven bioproduction processes Biotechnology Advances. https://doi.org/10.1016/j.biotechadv.2015.03.002

Patil, S. A., & Hägerhäll, C. (2012). Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems. 4, 159–192. https://doi.org/10.1007/s12566-012-0033-x

Prévoteau, A., Carvajal-Arroyo, J. M., Ganigué, R., & Rabaey, K. (2020). Microbial electrosynthesis from CO2: forever a promise? Current Opinion in Biotechnology, 62, 48–57. https://doi.org/10.1016/j.copbio.2019.08.014

Rabaey, K., & Rozendal, R. A. (2010). Microbial electrosynthesis — revisiting the electrical route for microbial production. 8. https://doi.org/10.1038/nrmicro2422

Ragsdale, S. W., & Pierce, E. (2008). Biochimica et Biophysica Acta Acetogenesis and the Wood – Ljungdahl pathway of CO 2 fi xation. 1784, 1873–1898. https://doi.org/10.1016/j.bbapap.2008.08.012

Roy, M., Yadav, R., Chiranjeevi, P., & Patil, S. A. (2021). Bioresource Technology Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis. Bioresource Technology, 320(PA), 124289. https://doi.org/10.1016/j.biortech.2020.124289

Song, T., Wang, G., Wang, H., Huang, Q., & Xie, J. (2019). Experimental evaluation of the influential factors of acetate production driven by a DC power system via CO 2 reduction through microbial electrosynthesis. Bioresources and Bioprocessing. https://doi.org/10.1186/s40643-019-0265-5

Songolzadeh, M., Soleimani, M., Ravanchi, M. T., & Songolzadeh, R. (2014). Carbon Dioxide Separation from Flue Gases : A Technological Review Emphasizing Reduction in Greenhouse Gas Emissions. 2014.

Taylor, P., Al-rawajfeh, A. E., & Al-amaireh, M. N. (2012). Desalination and Water Treatment The influence of CO 2 injection on the carbonate chemistry and scaling in multiple-effect distillers. November 2014, 37–41. https://doi.org/10.5004/dwt.2009.700

Wrighton, K. C., Agbo, P., Warnecke, F., Weber, K. A., Brodie, E. L., Desantis, T. Z., Hugenholtz, P., Andersen, G. L., & Coates, J. D. (2008). ORIGINAL ARTICLE A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. 1146–1156. https://doi.org/10.1038/ismej.2008.48

Zhang, T., Nie, H., Bain, T. S., Lu, H., Cui, M., Snoeyenbos-West, O. L., Franks, A. E., Nevin, K. P., Russell, T. P., & Lovley, D. R. (2013). Improved cathode materials for microbial electrosynthesis. Energy and Environmental Science, 6(1), 217–224. https://doi.org/10.1039/c2ee23350a

✧ Appendix

Table S-1:
Composition of trace metal solution.

Chemicals	**(g/L)**
Nitrilotriacetic acid (dissolve with KOH; pH 6.5)	1.5
$Mg_2Cl_2.6H_2O$	3.0
$MnCl_2.2H_2O$	0.5
NaCl	1.0
$FeCl_2$	0.1
$CoCl_2$	0.1
$CaCl_2.2H_2O$	0.1
$ZnCl_2$	0.1
$CuCl_2$	0.01
$AlCl_3.6H_2O$	0.01
H_3BO_3	0.01
$Na_2MoO_4.2H_2O$	0.01

Table S-2:
Composition of vitamin solution.

Chemical	**mg/L**
Sodium ascorbate	10
Biotin	4
Folic acid	4
Pyridoxine hydrochoride	20
Thiamine hydrocloride	10
Riboflavin	10
Nicotinic acid	10
DL-calcium pantothenate	10
Vitamin B12	0.2
p-aminobenzoic acid	10
Lipoic(thioctic) acid	10
Myo-inositol	10
Choline chloride	10
Niacinamide	10
Pyridoxal hydrochloride	10

Table S-3: Composition of tungstate-selenium solution

Solution	**mM**
Na_2WO_4	0.1
Na_2SeO_3	0.1
NaOH	20