Assessment of microbial electrosynthesis technology for utilizing unpurified CO_2 from industrial sources

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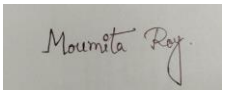
Dedicated

to my parents

Declaration

The work presented in this thesis 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 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 acknowledgment of collaborative research and discussions. This thesis is a bona fide record of my original work, and all sources listed within have been detailed in the bibliography.



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

Dr. Sunil A. Patil

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List of Publications

Articles in peer-reviewed international journals

- **Roy M.,** Yadav R., Chiranjeevi P., Patil SA. Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis. Bioresource Technology. 2021, 320:124289. doi.org/10.1016/j.biortech.2020.124289
- **Roy M.,** Yadav S., Patil SA. Biogas Upgradation Through CO_2 Conversion Into Acetic Acid via Microbial Electrosynthesis. Frontiers in Energy Research. 2021, 9:723. doi.org/10.3389/fenrg.2021.759678
- **Roy M.,** Aryal N., Zhang Y., Patil SA., Pant D. Technological progress and readiness level of microbial electrosynthesis and electro fermentation for carbon dioxide and organic wastes valorization. Current Opinion in Green and Sustainable Chemistry. 2022, 35:100605. doi.org/10.1016/j.cogsc.2022.100605
- **Roy M.,** Saich M., Patil SA. Scalability of microbial electro-acetogenesis process for biogas upgradation: performance and techno-economic assessment of a Litre-scale system Energy & Fuels. 2023, 37(20):15822-31. doi.org/10.1021/acs.energyfuels.3c02312
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Other articles

- **Roy, M.** and Das, S. Microbial Electrosynthesis: A Biobased Pathway for the Production of Value- Added Chemicals Through Carbon Sequestration. *In* Microbial Electrochemical Technologies: Fundamentals and Applications. 2023, 1, 361-379. Publisher: Wiley doi.org/10.1002/9783527839001.ch15

Patent

Indian Institute of Science Education and Research, Mohali, Patil S., **Roy M**. 2022. A process for increasing the calorific value of the biogas and system thereof. Indian Patent Application No. 202211025653

Synopsis

Chapter 1: Introduction

The catastrophic impacts of rising carbon dioxide (CO_2) concentrations in the atmosphere, including global warming and climate change, pose a severe threat to humankind. Various strategies, including carbon capture and utilization (CCU) approaches, are considered to address this problem and reduce the overall carbon footprint. CCU advocates preventing CO_2 emissions by capturing and utilizing or converting them into valuable products. Recycling CO_2 via CCU is highly desired as it offers a sustainable platform for producing chemicals and fuels and helps to close the linear carbon use cycle, thereby contributing to the transition from a petroleum-based chemical production industry to a more sustainable CO_2-based industry. Though several CCU technologies are under investigation, and the range of CO_2-derived products is expanding rapidly, multi-carbon and high-value compound production is still challenging, and large-volume CO_2 conversion is currently limited. The atmospheric CO_2 is too diluted to be used as a carbon feedstock via CCU without concentrating it. The flue gases emitted from industrial point sources are rich in CO_2 and thus need to be managed appropriately before their emission. By utilizing CO_2 directly from these point sources, a concentrated CO_2 stream can be obtained with minimum or no transportation and purification costs, which is highly desired to develop cost-efficient technologies. However, very few attempts have been made to test the suitability of unpurified industrial CO_2-rich gases for chemical production via different CCU technologies.

Among others, microbial electrosynthesis (MES) is an emerging CCU technology. It is an electricity-driven bioproduction process from CO_2 using microbial catalysts in bioelectrochemical systems. Significant progress has been made in assessing different electrode materials, the applicability of mixed and pure microbial catalysts, reactor designs, and the influence of operational conditions, continuous flow mode and hydraulic retention time, energy application, and media composition on the performance of MES. Since most research on MES from CO_2 has been conducted with pure gas or bicarbonate, the feasibility of utilizing industrial gases remains barely investigated. To this end, this doctoral thesis aimed to assess the feasibility of MES technology for producing chemicals from unpurified CO_2 from industrial sources. Both pure and enriched mixed chemolithoautotrophic microorganisms capable of CO_2

fixation via the Wood-Ljungdahl pathway were used for this purpose. The thesis work involved three key objectives: (i) Screening of different industrial gases for bioproduction via gas fermentation, (ii) Assessment of microbial electrosynthesis technology for acetic acid production with brewery CO_2, and (iii) Testing and scalability assessment of microbial electrosynthesis for biogas upgradation. These are outlined as specific chapters in the subsequent sections.

Chapter 2: Screening of different industrial gases for bioproduction via gas fermentation

First, different CO_2-emitting industries were identified according to the CO_2 content and accessibility near the Mohali region, Punjab (India). These include breweries, biogas plants, steel processing units, sugar mills, and incineration plants. The gas samples were collected using a customized set-up consisting of a 6 L steel canister, moisture and particulate matter traps, gas impermeable tubings, and a vacuum pump. The brewery gas contained a maximum CO_2 concentration (97.91± 1.52 %) among all industrial gas samples. Biogas contained mainly CH_4 (62 ± 7%) and CO_2 (28 ± 5%). In these two sources, the amount of O_2 was lower than 1 %. The gas samples from sugar mill, steel, and incineration plants contained a higher amount of O_2 (≥ 11 %) and a lower amount of CO_2 (≤12 %) besides other components such as NOx and SOx. The pure (*Clostridium ljungdahlii*) and *Acetobacterium*-dominated enriched mixed cultures were tested with H_2 and these industrial CO_2-rich gases as energy and carbon sources, respectively, for growth and organics (C1-C4 acids and alcohols) production via gas fermentation. Among all, considerable microbial growth and acetic acid production were observed only with brewery CO_2 and biogas. For instance, with brewery CO_2, the enriched mixed and pure cultures produced 1.3 ± 0.03 and 0.43 ± 0.02 g/L acetic acid, respectively. Among the microbial cultures, *C. ljungdahlii* was more susceptible to the impurities in the industrial CO_2 than the mixed culture. The other three gas samples containing a high concentration of O_2 cannot be used for bioproduction without purification since O_2 impacts the growth of anaerobic microbial cultures. Brewery CO_2 and biogas contained low impurities and O_2 that showed no significant detrimental effect on microbial growth. Hence, further work was conducted with brewery CO_2 (chapter 3) and biogas (chapter 4).

Chapter 3: Assessment of microbial electrosynthesis technology for acetic acid production with brewery CO_2

It involved the proof-of-concept demonstration and process optimization for brewery CO_2 conversion into acetic acid as two focused activities elaborated below.

3.1. Unpurified brewery CO_2 utilization through microbial electrosynthesis for the production of acetic acid

The proof-of-concept MES experiments were performed in H-shaped double-chambered reactors (working volume 0.25 L) housing graphite plate cathode, mixed metal oxide-coated titanium anode, and Nafion 117 proton exchange membrane. Both enriched mixed and pure cultures could utilize brewery CO_2 as a carbon source for growth and acetic acid production at an applied $E_{cathode}$ of − 1 V and − 1.2 V (vs. Ag/AgCl) via the chronoamperometry technique. A maximum acetic acid titer of 1.8 ± 0.21 (produced at 0.26 ± 0.03 g/L/d rate with 84 ± 13 % coulombic efficiency; CE, and at 2.8 V E_{cell}), and 1.1 ± 0.02 g/L (produced at 0.138 ± 0.004 g/L/d rate with 42 ± 14 % CE, and at 3.5 V E_{cell}) was achieved by enriched mixed and *C. ljungdahlii* cultures, respectively. These findings suggest that unpurified brewery gas can be used ‒as is‖ to produce acetic acid via the MES process. Microorganisms in the cathode chamber improved electrocatalysis by lowering the overpotential for H_2 evolution reaction and enhancing electric current draw. The SEM imaging of, for instance, mixed culture biocathodes confirmed the uniform biofilm growth at the cathode surface. The enriched mixed culture outperformed the pure culture in terms of production efficiencies due to its diverse microbial communities, as confirmed by microbial community analysis via 16s rRNA amplicon sequencing. *Acetobacterium* was one the most dominant genera (21 % relative sequence abundance) in the MES cathode chamber. This known acetogen can fix H_2 and CO_2 into acetic acid via the Wood-Ljungdahl pathway. *Pseudomonas spp.* (26 %), known to facilitate H_2 production, were also present in the reactors. *Desulfovibrio* detected at 9% abundance can facilitate electron transfer and the production of H_2 and formate through the activity of cytochromes, hydrogenases, and formate dehydrogenase enzymes. These two genera might have played a role in improving hydrogen electrocatalysis at the cathode. *Sulfurospirillum*, a microaerophile (capable of scavenging remnants of O_2), was another dominant genus (13 %) in the cathode chamber. Though they can oxidize acetic acid to scavenge O_2, their presence in the reactor aided in sustaining anoxic conditions. This proof-of-concept demonstration work on brewery CO_2 was followed by process optimization with the better-performing and resilient enriched mixed microbial culture (section 3.2).

3.2. Brewery CO_2 conversion into acetic acid at an optimized set of microbial electrosynthesis process parameters

To improve the overall acetate productivity from brewery CO_2, the key operational parameters were optimized in a cubical two-chambered reactor (working volume 0.5 L) in which the distance between the electrodes, the electrode-to-volume ratio, and electrode-to-membrane ratios were improved compared to the H-shaped reactor. The same enriched mixed microbial culture and electrode materials were used in this reactor, and different gas feed rates (viz., 0.9, 0.7, 0.4 and 0.3 L/d) and $E_{cathode}$ (viz., −1.2, −1.0 and −0.8 V (vs. Ag/AgCl) were tested. The influence of catholyte recirculation in the cathode chamber was then tested for further process enhancement. At an optimized $E_{cathode}$ of – 1 V, the mixed culture produced up to 6.6 g/L acetic acid from brewery CO_2 fed continuously at a feed rate of 0.7 L/d. It was achieved at 85 ± 23 % and 35 ± 14 % CE and energy efficiencies (EE). Higher gas feed rates led to the swift removal of the H_2 generated in the cathode from the system. It impacted the performance of microbes that reduce CO_2 by using H_2 as their energy source. On the other hand, an excess amount of H_2 (41 % CE) remained underutilized at lower applied cathode potentials. Therefore, an optimum trade-off between the gas flow rate and applied cathode potential is essential for efficient H_2-mediated production. With the optimized set of conditions, enhancement in acetic acid titer was achieved without hampering the production efficiencies of the overall system. With the catholyte recirculation loop in reactors, the acetic acid production enhanced further to 7.6 ± 0.65 g/L at 92 % CE and an E_{cell} of 2.9 V at the same optimized conditions. The improved productivity can be attributed to the better mixing and retention of CO_2 and H_2 in the MES system. Overall, the findings of this chapter confirm the applicability of MES technology for utilizing unpurified brewery CO_2 for bioproduction.

Chapter 4: Testing and scalability assessment of microbial electrosynthesis for biogas upgradation

This chapter pertains to assessing the applicability and scalability of the microbial electrosynthesis process for biogas upgradation.

4.1. Assessment of microbial electrosynthesis for biogas upgradation through CO_2 conversion into acetic acid

Biogas has tremendous potential as a sustainable alternative energy source, but its calorific value is significantly reduced by the presence of CO_2, making it unsuitable for

use as fuel. Therefore, to boost CH_4 content, CO_2 must be removed beforehand. The scrubbed CO_2 is removed and not used in the existing energy or chemical-intensive biogas upgradation processes. Hence, MES, which allows CO_2 conversion, was tested as an alternative approach for biogas upgradation. The same reactor, electrodes, and enriched mixed culture were used, as mentioned in 3.1, for biogas upgradation experiments at different feed rates. The methane concentration increased from 62% (in raw biogas) to 93 % (in upgraded biogas) at an $E_{cathode}$ of −1 V (vs. Ag/AgCl) and a biogas feed rate of 0.3 L/d as a result of CO_2 conversion into acetic acid (~ 3.4 g/L) by microorganisms. The lower biogas feed rates improved CH_4 content increment and acetic acid production. *Acetobacterium* and *Desulfovibri*o dominated both the bulk phase and biocathode in MES reactors. With this approach, the same biogas feed can produce two distinct products: CH_4 in the off-gas and acetic acid in the bulk phase. These results encouraged further evaluation of the scalability of the demonstrated process, as presented in the next sub-section.

4.2. Assessment of the scale-up and techno-economic feasibility of the microbial electrosynthesis process for biogas upgradation

The follow-up MES process validation for biogas upgradation was conducted with scaled-up two-chambered parallel-plate reactors (4 L). In the galvanostatically-controlled reactors, the influence of different reduction current densities (viz., − 0.33, − 0.5, and − 0.66 mA/cm^2) was tested. Methane concentration increased from 56 ± 2 % to 86 ± 6 % at a fixed current density of − 0.5 mA/cm^2 in the MES reactors operated continuously with biogas at a 1 L/d rate. At 2.6 V E_{cell}, acetic acid was produced at 82 ± 16 % CE and 36 ± 7 % EE. Along with efficient production and biogas upgradation, the CE and EE of the L-scale reactor were comparable to those of small-scale reactors, suggesting the feasibility of this process in scaled-up reactors without compromising the key performance indicators. A sensitivity-analysis-based primary techno-economic assessment was conducted to determine the practicality of this technology based on the operational costs. The process involved varying the acetic acid titer and cell voltage within a specific range while keeping all other input parameters constant. The techno-economic assessment revealed that this process is not economically feasible and requires significant cost reduction. These results demonstrate that, even in large-scale reactors, biogas upgradation via the MES process is possible without forfeiting

essential performance parameters, but the process is not economically viable with the reactor electrode and membrane materials used in this study.

Chapter 5: Conclusions and Future Perspectives

This thesis work sheds light on the applicability of MES technology for utilizing unpurified CO_2 from industrial sources. In particular, MES is suitable for utilizing biogenic CO_2 derived from anaerobic digestion and fermentation processes, which are readily available yet underexplored carbon sources. It is a significant and much-needed step towards developing a sustainable and economically appealing CCU platform for utilizing unpurified industrial CO_2. The key research outcomes and future perspectives are summarized below.

- The bioproduction using enriched mixed microbial culture comprising mainly *Acetobacterium* sp. was more promising from biogenic CO_2 sources, e.g., from the brewery and anaerobic digester, than other industrial sources containing more impurities. Impurities, such as NOx, SOx and O_2 are detrimental to microbial catalysts. Additional purification of other industrial gases (e.g., sugar mill, steel, and incineration plants) is required to enable their utilization via bio-based CCU approaches involving anaerobic microbial catalysts. Microbial culture enrichment or adaptation according to specific industrial CO_2 sources may be beneficial in dealing with the impurities and developing robust microbial communities for the desired purpose. Alternatively, additional purification or/ pre-treatment of these industrial gases (e.g., sugar mill, steel, and incineration plants) is required before capture to eliminate these impurities in order to enable their utilization via bio-based CCU approaches involving anaerobic microbial catalysts.
- Utilization of unpurified industrial CO_2 was successfully demonstrated via MES with both enriched mixed and pure *C. ljungdahlii* cultures. Due to its robustness and diverse functionality, the enriched mixed culture outperformed *C. ljungdahlii*. The optimization of operational parameters and media recirculation resulted in improved acetic acid production of up to 7.6 ± 0.65 g/L at 92 % CE and an E_{cell} of 2.9 V. The anodic reaction consumed more energy in the overall process. Future studies with brewery CO_2 should aim for the on-site demonstration of a scaled-up MES process for producing acetic acid and other valuable products with different microbial catalysts.

- MES allows effective biogas upgradation by converting CO_2 into acetic acid. The demonstrated process offers not one but two products from the same biogas feed: upgraded biogas with > 85 % methane content and acetic acid up to 3.65 ± 0.64 g/L at an E_{cell} of 2.6 ± 0.6 V. In all MES experiments, enhanced electrochemical parameters, such as lowered H_2 evolution potential and increased reduction current, suggested the role of microbes in improving the electrocatalysis process. Biogas upgradation was feasible even in large L-scale reactors without compromising the key MES process performance indicators, suggesting process scalability. However, the primary techno-economic analysis revealed that the process is not economically viable. Future studies should focus on lowering capital expenses by exploring alternative low-cost but efficient electrode and membrane materials.

Overall, the findings of this thesis add substantially to the understanding of the applicability of MES technology for utilizing unpurified industrial CO_2 for bioproduction. This work opens up the possibilities of using alternative CO_2-rich flue gases for bioproduction, providing a significant advantage over electrochemical or catalytic CCU technologies, which are prone to traces of impurities in industrial CO_2. Future research should consider developing scalable reactor designs and alternative low-cost but efficient reactor materials without compromising the key bioproduction process indicators. The anodic water oxidation reaction is the major energy-consuming process in MES. Alternative anode reactions, e.g., organics oxidation, wastewater treatment, and chlorine production, should thus be explored to make the overall process economically appealing.

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Chapter 1

Introduction

1.1 Global scenario of carbon dioxide (CO_2) emissions from point sources

Over the past century, there has been a significant rise in atmospheric CO_2 levels, primarily driven by various human activities. Since the onset of the industrial era, the amount of anthropogenic CO_2 released has increased rapidly. By the end of 2022, it had reached 36.8 gigatons (IEA 2022). Industrial emissions, in particular, stand out as one of the primary culprits, accounting for 30% of the total global anthropogenic CO_2 emissions that are culminating in concerns such as global warming and climate change (Leeson et al., 2017). To address this pressing issue, nations worldwide, both developed and developing, are coming together in international forums like the recently concluded COP26 to achieve carbon neutrality within the next few decades. By targeting emissions from industrial processes, the overall carbon footprint can be reduced significantly, thereby achieving carbon neutrality. This proactive approach is expected to not only help in curbing global warming and climate change but also foster sustainable industrial practices for a healthier planet.

Several industrial sources have been identified as significant contributors to CO_2 emissions (von der Assen et al., 2016, Bahman et al., 2023, Leeson et al., 2017). Table 1.1 highlights major industrial CO_2 emitters along with their respective contributions to CO_2 emissions. Prioritizing the capture and utilization of CO_2 directly from industrial point sources presents numerous benefits. These sources emit concentrated streams of CO_2, simplifying the carbon capture process and reducing costs compared to capturing CO_2 from diluted atmospheric sources. By leveraging these concentrated emissions, the necessity for expensive transportation and purification processes can also be minimized (Simonsen et al., 2024).

Table 1.1 indicates that coal power plants and the cement industry are the primary industrial contributors to CO_2 emissions, with the steel industry and oil refineries following closely behind. Despite not being the primary sources of anthropogenic CO_2 emissions, industries such as breweries and biogas plants make notable contributions due to the high CO_2 content in their gas effluents. Industrial CO_2 can be a valuable carbon source if reutilized through various Carbon Capture and Utilization (CCU) approaches. These are discussed further in the next section.

Table 1.1 Major CO_2 emitting point industrial sources.

Source	Contribution to total industrial emissions	Amount of CO_2 in flue gas	Reference
Coal Power plant	40 % (in total emissions from the energy sector)	12-15 %	Jakob et al., 2020, von der Assen et al., 2016
Cement Production	26 %	14-33 %	Plaza et al., 2020
Iron and steel industry	25 %	17- 35 %	IEA 2020, Lei et al., 2023
Refineries	6-10 %	10-20 %	IEA 2021, Jing et al., 2020
Pulp and paper industries	2 %	7-20 %	Dai et al., 2023
Breweries	< 1 %	> 95 %	Dessi et al., 2021, Shin and Searcy, 2018
Biogas plants (Anaerobic digesters)	< 1 %	30-50 %	Thiruselvi et al., 2021

1.2 Carbon capture and utilization (CCU) technologies for reducing carbon footprint

Primarily, there are two methods to deal with CO_2 emissions: carbon capture and storage (CCS) and carbon capture and utilization (CCU). These approaches have emerged as pivotal ones in mitigating greenhouse gas emissions, particularly CO_2. These encompass capturing CO_2 emissions at the source, repurposing captured CO_2 for various industrial applications, or securely storing it underground to prevent its release into the atmosphere. Through CCS methods, CO_2 originating from industrial sources is stored in potentially safer environments such as underground or depleted oil reservoirs (Cuéllar-Franca and Azapagic, 2015). However, the long-term implications of this approach remain uncertain. Therefore, the proposition of CCU has recently gained great interest throughout the globe as it provides one of the potential solutions to address the CO_2 issue. In CCU, CO_2 can be utilized directly for applications such as dry cleaning and enhanced oil recovery, or it can serve as a feedstock for generating organic compounds. Using CO_2 as feedstock advocates preventing CO_2 emissions into the atmosphere by capturing and converting them into valuable chemical compounds (Artz et al., 2018). A key driver of investment in CCU is its ability to secure a supply of fuels and commodity chemicals that have traditionally

relied on petrochemical and biomass feedstocks and the possibility to couple it with renewable energy storage via the Power-To-X approach (Gong et al., 2021). The utilization of CO_2 as feedstock involves both non-biological and biological approaches. Non-biological methods include chemical, electrochemical, and photochemical processes, each at varying technological readiness levels (Dziejarski et al., 2023). However, these methods primarily yield C1 compounds as their final products (Cai et al., 2024). The processes necessitate meticulous control of reaction conditions and the utilization of costly catalysts. Furthermore, due to the utilization of chemical catalysts, these processes face challenges such as catalyst irreversibility and susceptibility to catalyst poisoning from even small amounts of gaseous impurities (Luan et al., 2023).

Biological processes, such as algal cultivation, gas fermentation, and microbial electrosynthesis (MES), offer greater promise for CO_2-based production of chemicals (Mikulčić et al., 2019). These methods leverage biological processes to convert CO_2 into desired multicarbon products. They exhibit advantages over non-biological ones, including the potential for higher product diversity, reusability of biological catalysts, reduced reliance on finite resources, and greater environmental compatibility. Algal cultivation is among the leading CCU technologies, harnessing the photosynthetic capability of algae and cyanobacteria to capture CO_2 from various sources and convert it into valuable products (Figure 1.1a). Through algal cultivation, a large amount of CO_2 emissions can be mitigated while producing a range of products, including biofuels, animal feed, food supplements, pharmaceuticals, and bioplastics, at an industrial scale (de Morais et al., 2019). However, this process needs considerable water, arable land, and solar energy, besides costly downstream processing, which constrains its broad implementation. Another technology, gas fermentation, enables the conversion of CO_2 or CO into useful products with the help of anaerobic microbial catalysts under high-pressure conditions (Figure 1.1b) (Köpke and Simpson, 2020). This method is currently being implemented commercially by LanzaTech Pvt. Ltd. for ethanol production. However, in the case of gas fermentation, external hydrogen (H_2) or CO needs to be provided as an energy source for the microbes. This limitation can be circumvented in the MES process, as H_2 can be generated electrochemically within the system. It is the most direct way to deliver and store excess electricity in the form of value-added chemicals. This process primarily

converts energy into products rather than biomass. It demands less land and water and operates under ambient conditions, making it a promising and sustainable CCU approach. MES is currently a nascent technology in terms of industrial application compared to other biological and non-biological CCU approaches.

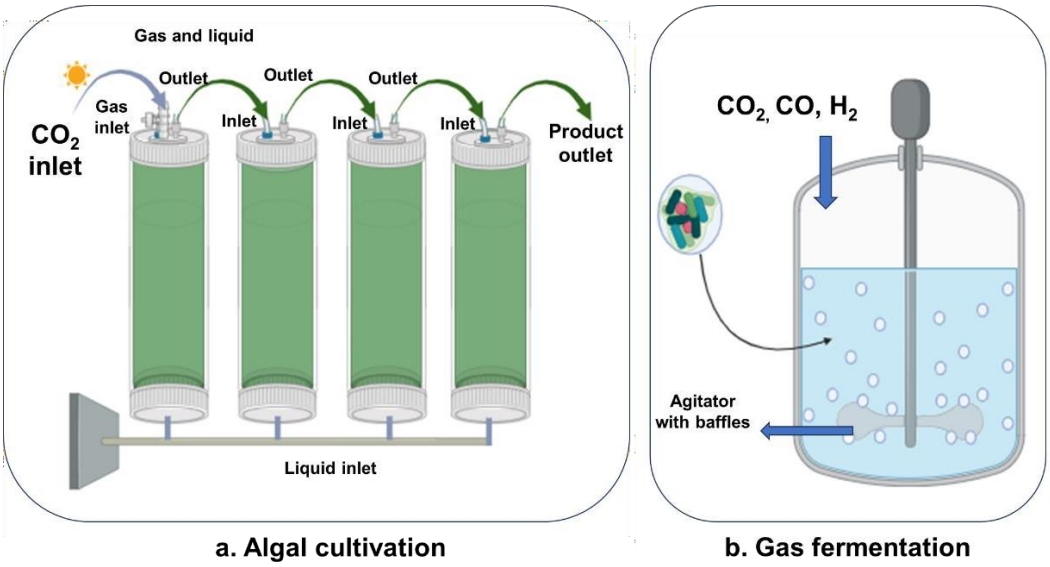

Figure 1.1: A schematic of algal cultivation (a) and gas fermentation (b) technologies.

1.3 Microbial Electrosynthesis (MES) for CO_2 utilization: process and state-of-the-art

1.3.1 MES process aspects

Microbial electrosynthesis (MES) is an emerging promising CCU technology for producing chemicals from CO_2. It operates at the nexus of microbiology and electrochemistry to convert CO_2 into reduced organic products (Rabaey and Rozendal 2010, Nevin et al., 2010). It offers the most direct way of supplying energy to the microorganisms via electrodes (Rabaey and Rozendal 2010, Lovley and Nevin, 2013). Usually, an MES system comprises two chambers: a cathode chamber and an anode chamber, separated by a proton exchange membrane (PEM). Within the abiotic anode chamber, typically, a water oxidation reaction occurs, yielding protons and electrons and releasing O_2 (Figure 1.2, Eq. 1). Subsequently, the protons migrate through the PEM from the anode chamber to the cathode one. With the electrons sourced from the anode via an external circuit, these protons can further be reduced to H_2 in the cathode chamber at a certain cathode potential (Eq. 2). The CO_2-fixing microbes, specifically electroautotrophs, can directly draw electrons from the cathode, or utilize

electrochemically-produced H_2 or other energy carriers as the energy source (Labelle et al., 2020) and fix CO_2 into, for instance, acetic acid (Eq. 3).

In situ H_2 production offers some advantages over its counterpart direct electron transfer-based process and other bioproduction process gas fermentation. By directly integrating H_2 production into the MES system, separate electrolyzers or external H_2 sources are avoided, simplifying process design and reducing operational complexity. Unlike gas fermentation, MES doesn't rely on external H_2 sources, enhancing its self-sustainability. Mass transfer limitations, common in large-scale reactors when supplying gaseous substrates to microorganisms, can be mitigated by in situ H_2 production through electrolysis, potentially leading to more efficient substrate utilization. Additionally, it enables continuous operation through continuous H_2 production without intermittent H_2 replenishment, which is particularly beneficial for industrial-scale production processes. However, in situ H_2 production through the water electrolysis reaction incurs additional energy requirements to the overall process. The extra costs associated with in situ H_2 production through electrolysis, including equipment, electricity, and maintenance, may impact the economic viability of MES. In addition to H_2-mediated production, direct electron transfer facilitated by microbial biofilms can also occur, wherein microbes directly acquire electrons from the cathode, utilizing them as their energy source. While this method leads to achieving greater energy efficiency and allows the retention of microbial biomass in continuous flow mode reactors, it typically results in a lower yield of organic production.

Anode reaction: $2H_2O = 4H^+ + O_2 + 4e^-$ (E_{anode}: 0.82 V vs SHE)* …(1)

Cathode reaction: $4H^+ + 4e^- = 4H_2$ (E_{anode}: − 0.41V vs SHE)* …(2)

Overall reaction: $2CO_2 + 4H_2 \rightleftharpoons CH_3COO^- + H^+ + 2H_2O$ (E_{cell}: 1.23 V vs SHE)*…(3)

*The potentials of denoted reactions at the anode and cathode electrodes are presented against the standard hydrogen electrode (SHE) under standard conditions (pH 7, pressure 1 bar, temperature 25 ± 5 °C).

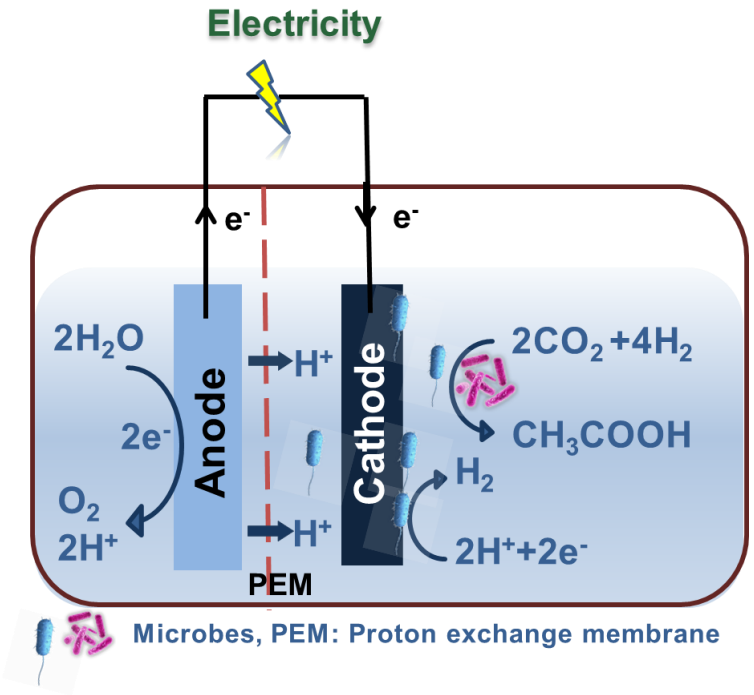

Figure 1.2: A schematic of a typical MES process showing anodic and cathodic reactions.

A plethora of microorganisms possess the ability to fix CO_2 via diverse metabolic pathways. Among these, the Wood-Ljungdahl pathway (or reductive acetyl-CoA pathway) stands out as one of the oldest metabolic pathways for reducing CO_2 into organic compounds (Eq. 3). Wood-Ljungdahl pathway is pivotal in various natural ecosystems, including anaerobic environments such as sediments, soils, and the gastrointestinal tracts of animals. In this pathway, CO_2 or CO is enzymatically reduced to acetyl-CoA, which is then subsequently converted to acetate (Figure 1.3) (Ragsdale, 2008). This process is typically carried out under anaerobic conditions, making it a key contributor to carbon fixation in anoxic environments. Acetogens, the chemolithoautotrophic microbes that utilize this pathway, are vital contributors to carbon fixation in oxygen-depleted environments. The acetogen that produces only one end product is known as a homoacetogen (e.g., *Acetobacterium woodii).* Some microbes, such as *Clostridium*, can produce more than one end product via this pathway. This pathway is significant not only for carbon fixation but also for its potential applications in biotechnology and bioenergy production. In this thesis, acetogenic microbes, both enriched mixed and pure cultures, were used as the biocatalysts for CO_2 fixation.

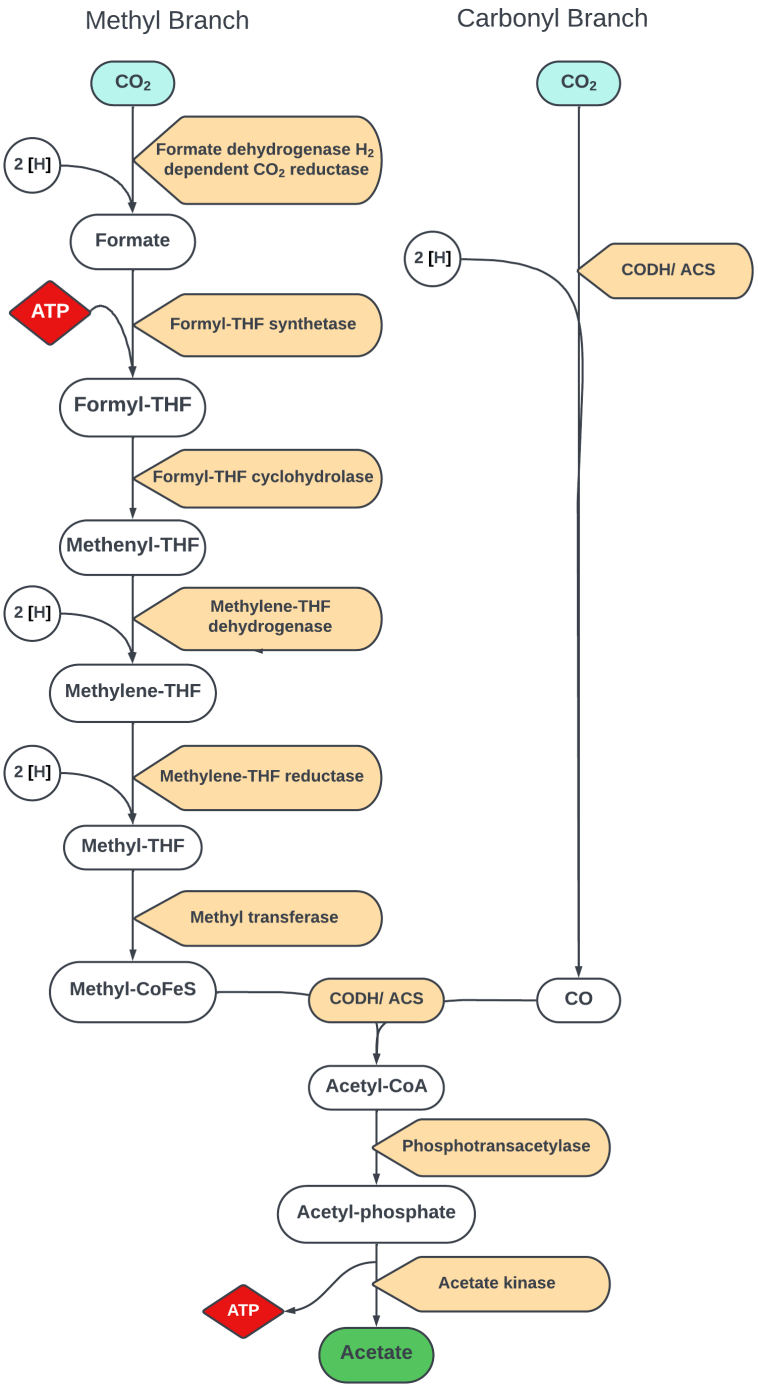

Figure 1.3: Schematic representation of the Wood-Ljungdahl pathway of CO_2 fixation. (Adapted with permission from Westerholm et al., 2016). Abbreviations: Carbon monoxide dehydrogenase (CODH), tetrahydrofolate (THF), acetyl-CoA synthase (ACS).

1.3.2 MES state-of-the-art

In the first proof-of-concept MES study, *Sporomusa ovata* was utilized as the biocatalyst, resulting in the successful synthesis of acetate and 2-oxobutyrate at a

potential of − 0.4 V vs. SHE (Nevin et al., 2010). Over the past few years, considerable advancements have been made in MES technology in terms of improving product spectrum, productivity and rates, conversion efficiencies, electrode materials, reactor designs, and testing different microbial catalysts. For example, though acetate remained the major product of the CO_2 reduction process via MES, the product range for MES expanded to longer carbon compounds like caproate and alcohols such as ethanol, isopropanol, and n-butanol as well as gaseous products like CH_4 (Geppert et al., 2019, Jiang et al., 2020, Claassens et al., 2019, Vassilev et al., 2019, Omidi et al., 2021). Both pure and enriched mixed inocula were investigated for this purpose. *Clostridium* and *Sporomusa* have been predominantly employed for the conversion of CO_2 into organic compounds (Nevin et al., 2010, Nevin et al., 2011, Zhang et al., 2013, Bajracharya et al., 2015). Enriched mixed cultures have also been utilized as biocatalysts in numerous studies (Jiang et al., 2013, Patil et al., 2015a, Bajracharya et al., 2017a). Enriched mixed cultures often demonstrated robust and superior performance compared to pure cultures, owing to the synergistic relationships among microbial consortia, wide availability, and ease of operation, ensuring long-term application as well as the economic viability and sustainability of the technology. However, pure cultures offer advantages in product selectivity and downstream processing and enable the possibility of enhancing production through genetic modifications. Though high coulombic efficiencies were reported in multiple studies (Xiao et al., 2020, Bajracharya et al., 2022, Song et al., 2022), the energy efficiency of the MES process remains lower (Prévoteau et al., 2022). One of the main goals of this bioelectrochemical technology was to utilize renewable energy sources to drive product synthesis reactions (Rojas et al., 2018a). In this scenario, advancement was made in recent years by demonstrating solar energy use to power the bio-electrochemical systems (BES) reactors (Xiao et al., 2020). Carbon-based electrodes represent the most commonly utilized cathode material in MES due to their biocompatibility, cost-effectiveness, and widespread availability (Jiang et al., 2013, Bajracharya et al., 2017b, Jourdin et al., 2018). To further optimize the process and enhance cathode-microbe interactions, various cathode surface modifications were implemented (Chen et al., 2016, Aryal et al., 2017, Chiranjeevi and Patil, 2020). For instance, the incorporation of carbon nanotubes (CNTs) and nickel nanowires was

shown to improve conductivity, biocompatibility, and electrostatic interactions, thereby increasing the productivity of MES from CO_2 (Zhang et al., 2013, Nie et al., 2013). Additionally, improved mass transfer was achieved through techniques such as chemical vapor deposition and electrodeposition of CNT on cathodes, along with the utilization of gas diffusion electrodes (Jourdin et al., 2014, Bajracharya et al., 2016).

Various reactor designs have been explored for MES as it is a critical aspect for assessing the scalability and real-world applications of the technology. The commonly used H-shaped double-chambered design, as documented in literature (Navin et al., 2010, Deutzmann and Spormann, 2017, Jourdin et al., 2016, and Mohanakrishna, et al., 2020) has some drawbacks, including high ohmic losses due to large electrode spacing, high energy consumption, low CO_2 mass transfer rates, and operational complexity in continuous mode (Krieg et al., 2019, Liu et al., 2023). To overcome such bottlenecks, parallel-plate two-chambered reactors were used in some studies where the distance between the cathode and anode chamber was reduced (Tahir et al., 2020). In long-term operation, these reactors allowed organic acids such as formic and acetic acids to pass through the PEM to some extent (up to 10 %). In situ extraction and recovery of products like acetic acid in a single modular reactor have thus been attempted (Das et al., 2021). Furthermore, to bring down the cost associated with the use of membranes, single-chamber, membrane-less reactors were investigated by some research groups (Li et al., 2019, Wang et al., 2021). Dual cathodes containing three-chamber reactors have been reported for MES (Vassilev et al., 2019). Recently, the highest acetic acid titer of 34.5 g/L has been achieved in a novel electro-H_2 bubble column reactor with an external hollow fiber membrane gas–liquid contactor (Cui et al., 2023). A few scale-up studies have been conducted on MES so far (Enzmann and Holtmann, 2019, Ceballos-Escalera et al., 2020, Zou et al., 2021, Shang et al., 2023). For example, CH_4 production was reported in 50 L volume reactors operated at −1.1 V vs Ag/AgCl cathode potential and inoculated with *Methanococcus maripaludis S2* (Enzmann and Holtmann, 2019). Though the CH_4 production rate was higher (11.7 mM/d) than most small-scale studies, the production was still not up to the mark, majorly due to the high internal resistance from the large reactor and electrodes. It was mainly due to the high internal resistance of the large reactor and electrodes. On

the brighter side, the energetic efficiency was considerably higher (27 %) when compared with the small-scale reactors (10 %) (Enzmann and Holtmann, 2019). A novel 20 L electrochemical continuous stirred-tank reactor was also reported to produce CH_4 at a high production rate and coulombic efficiency of 407.8 $L/m^2/d$ and 99.4 %, respectively (Shang et al., 2023). Achieving high production rates and efficiencies is crucial for the practical application of bioelectrochemical processes in converting CO_2 into valuable fuels and chemicals. It should be noted here that all MES work and advancements in CO_2 utilization have been done or demonstrated with pure CO_2 or bicarbonate.

1.4 Status of industrial CO_2 utilization via bio-based CCU approaches

Utilizing CO_2 directly from industrial point sources offers a concentrated stream with minimal transportation and purification costs, making it attractive for developing economically viable CCU technologies (Mikulčić et al., 2019). However, industrial flue gases often contain contaminants like SOx, NOx, CO, fly ashes, O_2, and volatile organic compounds, necessitating significant gas cleaning and conditioning to prevent catalyst poisoning for non-biological CCU approaches (Pappijn et al., 2020). In this context, bio-based CCU methods such as algae cultivation, gas fermentation, and MES offer the potential to utilize industrial CO_2-containing gases directly without the need for purification or partial cleaning. Algae, for example, demonstrated the ability to utilize various industrial gases as carbon sources (Singh et al., 2016, Singh et al., 2019, Cheng et al., 2019, Cutshaw et al., 2020). For instance, Cutshaw et al. (2020) showcased the long-term utilization of power plant flue gas in a pilot-scale reactor. Yang et al. (2021) conducted techno-economic and environmental analyses, finding urea production to be economically advantageous compared to sulfur and methanol production from flue gases. Aslam et al. (2017) and Cheng et al. (2019) highlighted the possibility of CO_2 utilization and the importance of genetic modification and improvement of microalgal strains to withstand toxic impurities (such as SOx, NOx, and fly ashes) present in coal-fired flue gas.

In the case of gas fermentation, both pure and mixed microbial catalysts have shown promise for utilizing industrial CO_2 from various sectors like breweries and steel industries (Novak et al., 2021). The process has been scaled up to an industrial level

by LanzaTech, where industrial gases from the steel industry, refinery, and power plants are used to produce an array of value-added products. However, the suitability of MES for utilizing unpurified CO_2 for bioproduction compared to algae cultivation and gas fermentation remains to be assessed.

1.5 Research objectives

It is widely acknowledged that employing industrial CO_2 emissions "as is," or with minimal purification, is advantageous for the development of cost-effective and efficient CCU processes, including MES (Bajracharya et al., 2015, Jourdin et al., 2020, Das et al., 2020). As highlighted earlier, pure CO_2 or bicarbonate served as the sole carbon source in the MES studies so far. Hence, the feasibility of utilizing industrial CO_2 emissions directly through MES remains unknown. Considering the above-mentioned technological knowledge gap, the doctoral thesis sought to evaluate the viability of microbial electrosynthesis (MES) technology for producing acetic acid from unpurified industrial CO_2. To achieve this, the thesis work involved three key objectives: (i) Screening of different industrial gases for bioproduction via gas fermentation, (ii) Assessment of microbial electrosynthesis technology for acetic acid production with brewery CO_2, and (iii) Testing and scalability assessment of microbial electrosynthesis for biogas upgradation. These are outlined as specific chapters in the subsequent sections. Figure 1.4 displays the main research activities and workflow involved in accomplishing these thesis objectives.

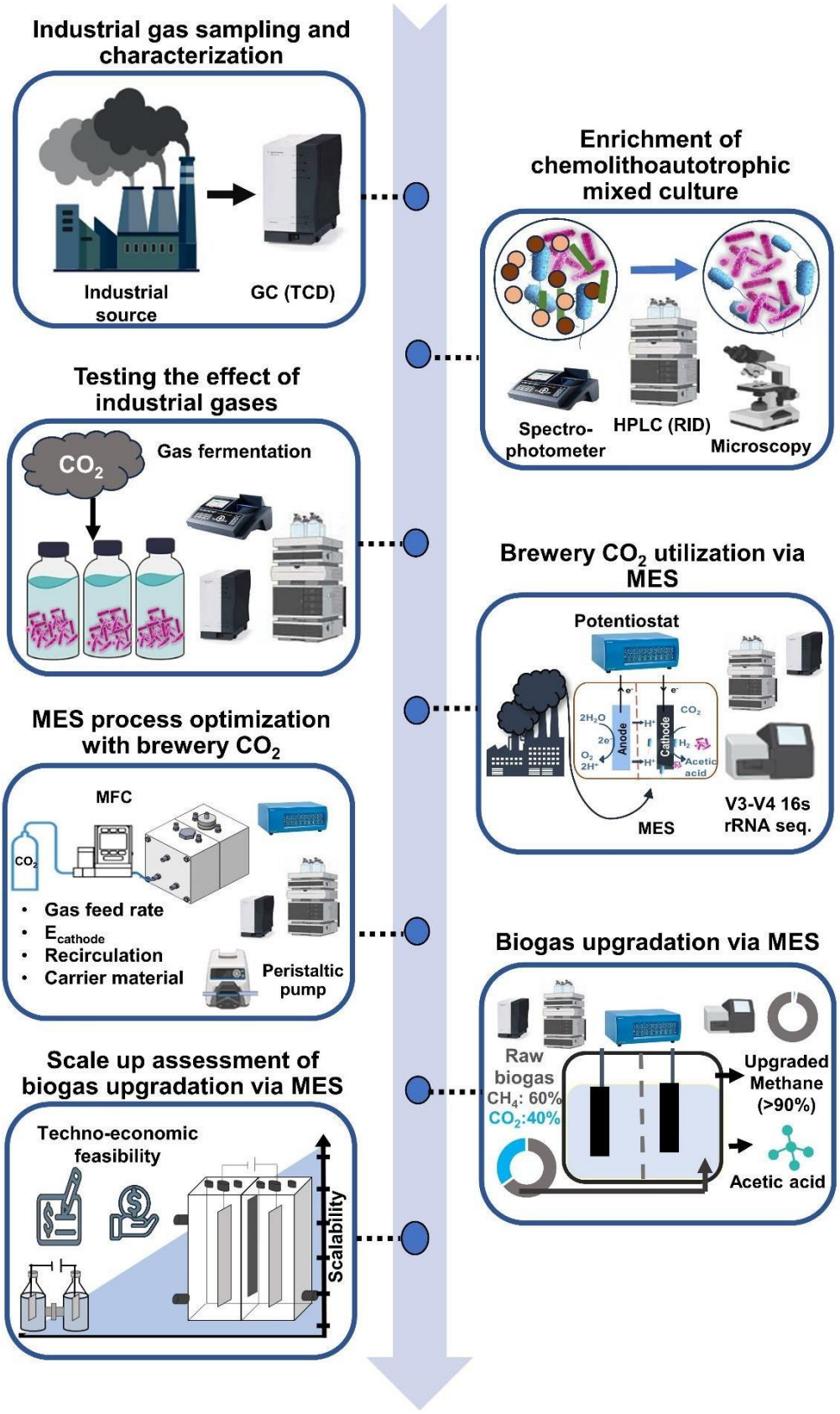

Figure 1.4: Schematic representation of the workflow and key research activities of this thesis work.

Chapter 2

Screening of different industrial gases for bioproduction via gas fermentation*

*Some of the contents of this chapter (particularly section 2.3.2.1: Brewery CO_2 as a carbon source) are adapted with permission from the following peer-reviewed journal publication:

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

2.1 Introduction

Cement and steel manufacturing industries, breweries, coal-powered power plants, and oil and gas production plants are the backbone of urbanization. The flue gases emitted from industrial point sources are rich in CO_2 and are a major source of pollution if not managed appropriately before their emission into the atmosphere. These point sources account for almost 30 % of all CO_2 emissions worldwide (Leeson et al., 2017). For instance, the share of cement and steel industries in the total anthropogenic CO_2 emissions is around 6 % and 4-5 %, respectively (Barker et al., 2009). The point sources offer the opportunity to get concentrated CO_2 for further valorisation compared to the atmospheric CO_2, which is diluted, requiring further capture and concentration processes. One of the prominent reasons for this constriction is the technical challenge of collection, transportation, and purification of CO_2 from different point sources (Mikulčić et al., 2019, Simonsen et al., 2024). By utilizing CO_2 directly from these point sources, a concentrated CO_2 stream can be obtained with minimum or no transportation and purification costs. Recycling CO_2 emissions offers not only a sustainable platform for producing chemicals and fuels but also helps to close the linear carbon use cycle and contributes to transitioning from a petroleum-based chemical production industry to a more sustainable CO_2-based chemical production industry.

Since non-biological CCU approaches are more susceptible to even trace amounts of toxic impurities, biological CCU ones are more beneficial for utilizing industrial gases –as is‖. Flue gases are already being utilized for bioproduction via algal cultivation and gas fermentation (section 1.4). Among the biological CCU processes, gas fermentation has emerged as a promising CCU approach. It is a process of converting gaseous substrates (like CO_2 and CO) to a spectrum of biobased products via microbial catalysts at high-pressure conditions (Liew et al., 2016, Redl et al., 2017). The microbes that are capable of CO_2 fixation via the Wood-Ljungdahl pathway (Figure 1.3) are utilized for this process. This approach is already being exploited at a commercial scale by Lanzatech Pvt. Ltd. Apart from purified syngas, unpurified gas emissions from steel, refinery, and power plants are also being used in this company for bioproduction (https://lanzatech.com) (Norouzi and Choubanpishehzafar, 2021, Harmon and Holladay, 2020). Though a few industrial flue gases are employed for gas fermentation, their effect on different chemolithoautotrophic cultures has not been

elaborately investigated. Therefore, in this thesis, different CO_2-rich industrial sources were first identified according to the CO_2 content and accessibility near the Mohali region, Punjab (India). After characterizing their gas composition, their influence on the growth of chemolithoautotrophic bacteria was tested in gas fermentation experiments.

2.2 Methodology

2.2.1 Industrial point sources and gas sampling

Depending on availability, gas samples from the following industrial sources were collected: brewery, anaerobic digester, steel processing, sugar mill, and incineration plants. The major CO_2-producing steps and their location are described in Table 2.1.

Table 2.1: Different industrial point sources and CO_2 production processes.

S. N.	**Industry**	**Name and Location**	**Process involved in producing CO_2**
1.	Brewery	Om Sons Marketing Pvt. Ltd, Bathinda	Fermentation (Barley grains)
2	Anaerobic digester	Phase 3, Baltana, Mohali; Biological treatment unit	Anaerobic digestion to treat domestic sewage
3	Steel processing plant	Aarti Steel Ltd. Ludhiana	Iron ore processing and steel making
4	Sugar mill	Morinda Sugar Mill Pvt. Ltd., Morinda, Punjab	Carbonation for purification
5	Incineration plant	Rainbow Industries Pvt. Ltd. Mohali	Incineration of o biomedical waste

A custom-made setup was used to collect gaseous emissions from these sources (Figure 2.1). It consisted of a canister (SilcoCan steel canisters with 3-PortSiltek-Treated RAVE with Gauge, Restek, United States), a vacuum pump (model N86 KT.45.18; KNF pump), Teflon tubing and connectors, filters, moister trap, and UPS (uninterruptible power source) for power supply. The canister was a round-shaped container of stainless steel (SS) with a provision (single opening) to collect and dispense the gas with external tubing. It was equipped with a control valve and pressure gauge to regulate the gas flow rate.

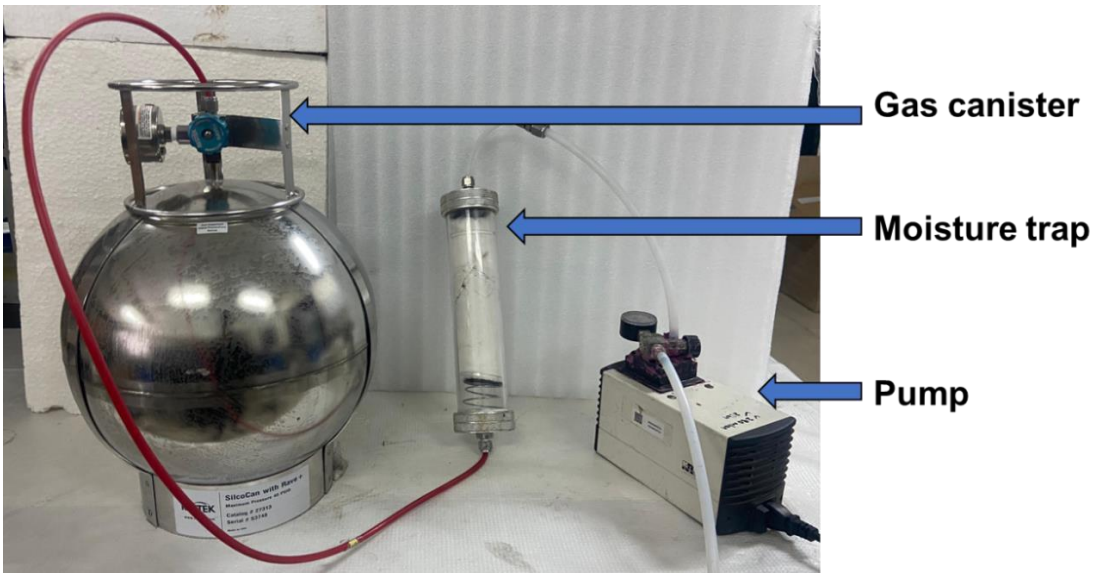

Figure 2.1: Set-up used for the collection of gaseous emissions from different point sources (Courtesy: Prof. Dr. Vinayak Sinha, IISER Mohali).

The vacuum pump helped in suctioning the gas at a flow rate of 8 L/min to fill the canister under the pressurized condition. It consisted of one suctioning port to suck the gas from the source and one outlet to fill the gas into the canister connected by the tubing. The vacuum pump was also equipped with a pressure gauge to indicate the pressure. All connectors for joining the tubes were of Teflon, which was an inert material that resists high temperature (>180 °C) and pressure (> 40 psi) conditions. One of the tubes was connected to a Teflon connecter with a PTFE (polytetrafluoroethylene) filter (0.2 μm) to filter the particulate dust matter from the raw gas source (to avoid choking the tube and corrosion of the canister). In order to reduce moisture content, a moisture trap consisted of an acrylic cartridge in which magnesium perchlorate (≥98.0 % pure, product no: 63095, Sigma-Aldrich) was packed in between two layers of cotton. Further, to restrict any particulate matter and traces of magnesium perchlorate from entering the canisters, double layers of Millipore filters (0.22 μm) were placed at both ends of the cartridge. A UPS was used to power the vacuum pump in the absence of the electrical points at the sampling sites. Before using the canister for gas collection, it was cleansed with liquid nitrogen, followed by vacuuming.

2.2.2 Industrial Gas Characterization

The collected gas samples were analysed to determine their composition by gas chromatography equipped with the thermal conductivity detector (GC-TCD, Agilent

490 Micro GC). There GC has three channels: Channel 1: Column- Molecular sieve, Carrier gas- Argon, detects Hydrogen gas; Channel 2: Column- Molecular sieve, Carrier gas– Helium, detects – Oxygen, Nitrogen, Carbon monoxide and Methane; Channel 3: Column- Pora plot U, Carrier gas– Helium, detects – Carbon dioxide and Hydrogen sulphide. The trace gas analysis was performed as mentioned elsewhere (Sinha et al., 2014, Kumar et al., 2020).

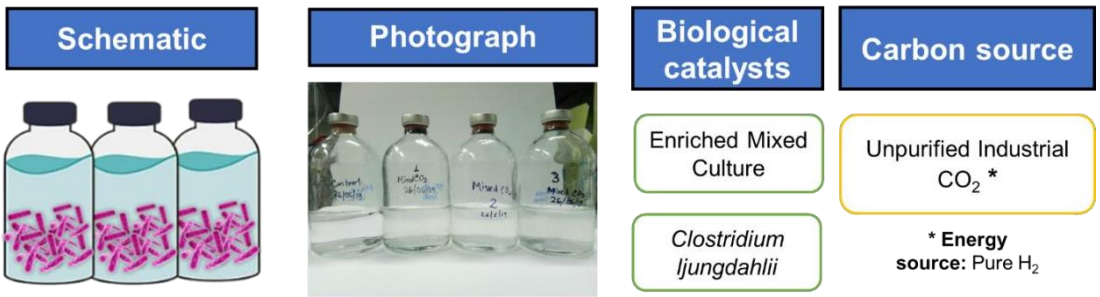

Figure 2.2: Schematic and digital image of the serum flasks used for the gas fermentation experiments with different microbial cultures and carbon sources.

2.2.3 Effect of CO_2 purity level on bioproduction via gas fermentation

The gas fermentation experiments conducted in serum flasks (100 mL capacity) were aimed at checking whether the chemolithoautotrophic microorganisms can grow by utilizing CO_2 in the presence of other gases and trace impurities in the gaseous emissions of various industrial sources (Figure 2.2). Two microbial cultures, namely, a pure strain of *Clostridium ljungdahlii* and an enriched mixed culture, were used for this purpose. All experiments were conducted under anaerobic conditions and at least in triplicates, as elaborated in sections 2.2.3.1 and 2.2.3.2. For comparison, with positive control for substrate conditions, these cultures were grown under autotrophic conditions using either bicarbonate or pure CO_2 and H_2 as carbon and energy sources, respectively.

2.2.3.1 Enriched chemolithoautotrophic mixed culture

The effluent from an anaerobic digester (AD) was used as the inoculum source for enriching chemolithoautotrophic microorganisms. Before use, it was sieved to remove cores and inert material and washed thrice with phosphate buffer. The suspension was then used as an inoculum source for the enrichment of anaerobic CO_2-fixing microorganisms. The enrichment experiments were conducted in a minimal medium

containing K_2HPO_4 (5.35 g/L), KH_2PO_4 (2.62 g/L), NH_4Cl (0.25 g/L), KCl (0.5 g/L), $CaCl_2.2H_2O$ (0.15 g/L), $MgCl_2.2H_2O$ (0.6 g/L), Trace Metal Solution (1mL), selenium – tungstate solution (1 mL), vitamin solution (2.5 mL), 2-bromo-ethanesulfonate (6.4 g/L), $Na_2S.9H_2O$ (0.3 g/L), L-cysteine (stock: 0.5 g/L), and resazurin (stock: 1 g/L). It was supplemented with CO_2 gas as the sole carbon source and H_2 as the sole electron source. To make medium anaerobic, buffer components (K_2HPO_4, KH_2PO_4, NH_4Cl, KCl) along with 2-bromoethanesulfonate (to suppress the methanogenic group) and 1 mL/L of resazurin (redox indicator for monitoring anaerobic conditions which turn pink in the presence of oxygen or oxidized condition) were added to the distilled water, and the medium was then boiled and cooled down under N_2 gas sparging conditions. The medium pH was adjusted to 7.0. Later, 40 mL of this buffer medium was transferred into 100 mL serum bottles under the N_2 gas condition to eliminate O_2 intrusion during media transfer. The medium in serum bottles was sparged with the same gas for 10 minutes and then sealed using butyl rubber stoppers and aluminium crimp seals. These bottles were then subjected to at least 20 gassing-degassing cycles (addition of N_2 gas to create overpressure and remove gases to create vacuum) to ensure anaerobic conditions, followed by autoclaving. Other medium components such as L-cysteine, vitamins, and trace metals were prepared separately under anaerobic conditions and added to the serum bottles via filter sterilization (0.22 µm membrane filters) aseptically. Microbial inoculation to catholyte was done under anaerobic conditions. After inoculation, Na_2S was added as the reducing agent in the serum bottles, and headspace was filled with filtered (with 0.2µm filters) pure H_2:CO_2 (80:20) at 1.3 ± 0.1 bar pressure. In the case of the industrial gases, pure H_2 (99.999% purity and filtered with 0.2µm filters) was added separately at 1.3 ± 0.1 bar pressure at the beginning of the experiment along with industrial CO_2, maintaining an H_2:CO_2 ratio of 4:1. By doing so, H_2 and industrial CO_2 were provided as the sole electron donor and carbon source, respectively. The serum bottles were incubated at 28 ± 2 °C under static conditions. The enrichment was followed by monitoring growth (OD_{600} analysis) and organics production in the bulk phase. The active enriched inoculum was maintained under the same conditions.

The experiments with industrial gases were conducted by following the same conditions mentioned above for the enrichment process. L-cysteine was eliminated in this experiment as it can contribute some amount of carbon to the gas fermentation

setup. For experiments with industrial gases, pure H_2 was used as the sole energy source and thus added to the headspace of serum bottles. The biotic and abiotic control experiments were conducted simultaneously. In the case of abiotic control, the buffer medium was supplemented with the H_2:CO_2 (80:20) gas mixture but was not inoculated with the microbes. Abiotic control was carried out to specify the contribution of microbial catalysts. In biotic control, the buffer medium was inoculated with an enriched microbial culture, but H_2:CO_2 gas was not added to avoid any carbon and energy source. It was to confirm that apart from H_2, no other energy sources were available in the system. In all experiments, the headspace gas pressure was maintained at 1.3 ± 0.1 bar.

2.2.3.2 Pure culture: Clostridium ljungdahlii

Clostridium ljungdahlii is a gram-positive, obligate anaerobic, and non-pathogenic bacterium which utilizes CO_2 via the Wood-Ljungdahl pathway using hydrogen as an electron donor or energy source. It is a well-studied acetogen. Due to its ability to form mainly acetic acid from CO_2 at neutral pH conditions, it was chosen for these experiments. The lyophilized culture of *Clostridium ljungdahlii* 13528 was obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). It was revived according to the standard protocol of DSMZ. The ampule of lyophilized *C. ljungdahlii* was opened anaerobically and aseptically in the biosafety cabinet under continuous N_2 flushing conditions. The lyophilized culture was dissolved using a buffer solution as per the DSM 879. The mixture was then transferred to the serum bottles (100 mL) containing 40 mL of DSM 879 medium consisting of NH_4Cl (1 g/L), KCl (0.1 g/L), KH_2PO_4 (0.1 g/L), $MgSO_4.7$ H_2O (0.20 g/L), NaCl (0.8 g/L), Trace element solution (10 ml/L), Yeast extract (1.00 g/L), Na-resazurin solution (0.1 % w/v) (0.50 ml/L), $NaHCO_3$ (10.00 g/L), vitamin solution (10.00 ml/L), L-Cysteine-HCl. H_2O (0.30 g/L), $Na_2S.9H_2O$ (0.30 g/L). The same approach was taken to prepare the serum bottles, as mentioned in 2.2.3.1. Care was taken to avoid contamination of the pure culture through filter sterilization of stock solutions. The serum bottles containing growth medium and lyophilized cells were then incubated at 37 °C for 5-6 days for reviving the culture. The growth was tracked by monitoring OD at 600 nm (UV-vis spectrophotometer). The – 80 °C stock and working cultures were prepared from the revived culture.

The same experiments with different gases (as described in the case of enriched mixed culture) were followed with the pure culture. In this case, L-cysteine and yeast extract were eliminated from the CO_2 utilization experiments.

2.2.4 Analysis and calculations

Headspace gas and bulk phase sampling from the serum bottles was done at regular intervals. It was followed by gas composition analysis by GC-TCD and organic acids and alcohols (C1-C4) analysis by High-performance liquid chromatography (HPLC) (Agilent 1260 Infinity II, RID Detector, Hiplex H column, 5 µM H_2SO_4 as mobile phase, flow rate 0.5 ml/min, Temperature 50 °C) to evaluate the efficiency of microbes to grow and produce organics from industrial CO_2 and H_2. The microbial growth in the bulk phase was monitored by analysing OD at 600 nm (Photo-lab 7600 UV-VIS spectrophotometer) and pH by pH meter (Oakton PC2700). Product titer and production rates were calculated according to protocols described elsewhere (Patil et al., 2015b)

2.3 Results and Discussion

2.3.1 Composition of different industrial gases

Table 2.2: Composition of different industrial gaseous emissions.

Gases (%) / ***Source***	***O_2***	***N_2***	***CH_4***	***CO_2***	***H_2S***	***Other compounds in trace amounts***
Incineration plant	3 ± 0.05	79 ± 3	--	2 ± 2	--	NOx, SOx, N_2O, CO, water vapours
Biogas plant	0.16 ± 0.35	4.84 ± 1.34	65.47 ± 10.97	24.05 ± 5.07	0.154 ppm	NH_3, CO, H_2
Brewery plant	0.356 ± 0.1	1.72 ± 0.70	--	97.91 ± 1.52	--	Hydrocarbons isobutanol, methanol, ethanol
Sugar mill	18 ± 0.01	72 ± 5	0.02 ± 0.004	5.5 ± 0.3	0.001	NOx, SO_2, water vapours
Steel processing plant	11 ± 0.4	71 ± 0.1	--	12 ± 0.3	--	NOx, Sox, VOC and water vapours

The sum of percentages of gases containing water vapors did not reach 100%.

The composition of gaseous emissions obtained from five different point sources is presented in Table 2.2. The gas matrix in the analysed samples is well in agreement with the data obtained from the source industries. Based on the GC data, it is evident that brewery gas effluent consists of a maximum CO_2 concentration among all the samples analysed. It does not contain any impurities at high levels. Biogas contains mainly methane and CO_2. In these two sources, the amount of O_2 was lower than 1 %. The gas samples from sugar mill, steel, and incineration plants contained a higher amount of O_2 (≥ 11 %) and a lower amount of CO_2 (≤ 12 %) besides other components such as NOx and SOx. The gases in which water vapours wwere present, the total percentage did not reach 100%. The presence of a high amount of oxygen is detrimental to the growth of anaerobic chemolithoautotrophic microorganisms.

2.3.2 Bioproduction from different industrial CO_2 sources

2.3.2.1 Brewery CO_2 as a carbon source

As the brewery CO_2 has the maximum amount of CO_2, it was fed as the sole carbon source to the pure as well as the enriched mixed culture, and for comparison, the same cultures were provided with other conventional carbon sources like bicarbonate and pure CO_2. As CO_2 fixation using H_2 is directly linked to growth and organics production (Ragsdale, 2008), the bulk samples collected at different time intervals were monitored for these parameters. The major organic acid produced was acetic acid in all experimental conditions with the enriched mixed and *C. ljungdahlii* cultures. When pure CO_2 was provided as a carbon source, the enriched mixed microbial culture entered the log phase of the growth cycle immediately (Figure 2.3). However, it took > 2 days to enter the log phase with brewery CO_2 (Figure 2.3a). A slightly longer lag phase might be due to the time required for microbial acclimatization to some impurities present in the brewery CO_2. The maximum acetic acid titer achieved by the mixed culture was, however, higher with the brewery CO_2 (1.3 ± 0.03 g/L produced at a rate of 0.185 ± 0.004 g/L/d) than the pure CO_2 feed (1 ± 0.09 g/L; 0.133 ± 0.011 g/L/d) (Roy et al., 2021a). The reasons for this observation are not known but might be attributed to a possible boost to microbes by some

impurities present in the brewery CO_2 that probably acted as additional nutrient sources.

In contrast, *C. ljungdahlii* performed better with pure CO_2 compared to the brewery CO_2 (Figure 2.3b and 2.3d). It might be because of the possible adverse effect of some impurities present in the brewery gas on the pure culture. In this case, acetic acid production reached 0.62 ± 0.17 g/L (0.1 ± 0.02 g/L/d) and 0.43 ± 0.02 g/L (0.061 ± 0.001 g/L/d) with pure and brewery CO_2, respectively (Roy et al., 2021a).

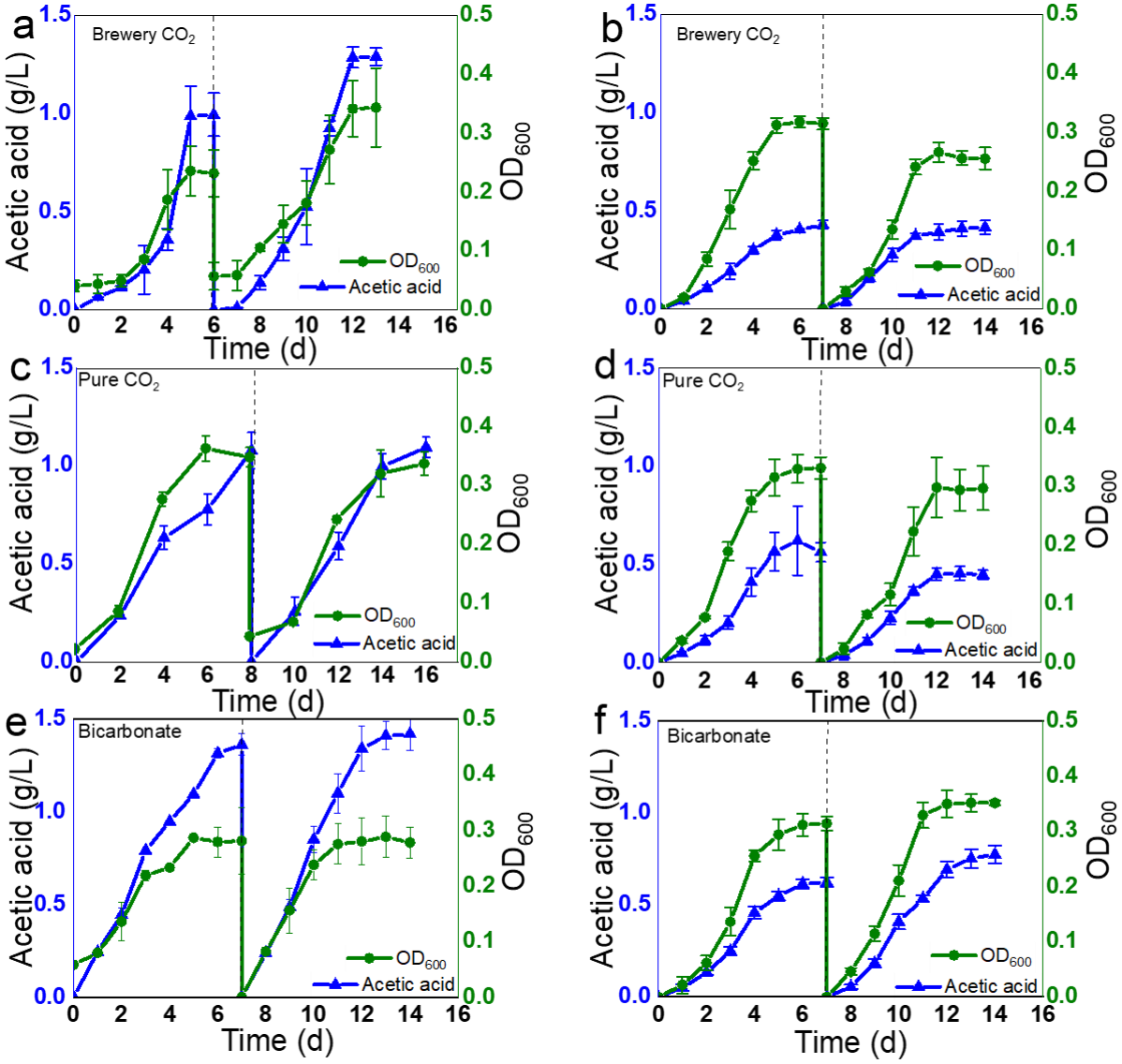

Figure 2.3: Acetic acid production and microbial growth (as OD_{600}) profiles of the enriched mixed (a, c, and e) and *C. ljungdahlii* (b, d, and f) cultures in batch gas fermentation experiments with pure CO_2, brewery CO_2, and bicarbonate. The dotted line in all graphs indicates the start of the second batch cycle.

As bicarbonate was readily available as a carbon source for microbes, an immediate start-up in growth and better acetic acid production were observed in the case of both pure and enriched mixed cultures (Figure 2.3e and 2.3f). Acetic acid production of 1.4 ± 0.28 g/L (0.2 ± 0.04 g/L/d) and 0.77 ± 0.05 g/L (0.11 ± 0.007 g/L/d) was achieved by the mixed and pure cultures, respectively. According to the OD data acetic acid production was linked well with microbial growth in all cases (Figure 2.3).

These experiments revealed the capabilities of both mixed and pure microbial cultures to efficiently utilize the brewery CO_2 and their tolerance to low levels of impurities present in it. The gas fermentation data indicated that the mixed culture outperformed *C. ljungdahlii* in terms of growth and organic acid production with brewery CO_2. Therefore, further gas fermentation experiments with other flue gases were conducted with the enriched mixed culture.

2.3.2.2 Biogas, sugar mill, steel processing industry, and incineration plant gases as carbon sources

Biogas also had a considerable amount of CO_2 compared to that of O_2, along < 1 % H_2S. The enriched mixed culture was able to readily utilize the CO_2 portion of the biogas as the sole carbon source. As a result, growth and acetic acid production were visible from the very beginning of the batch cycle (Figure 2.4a). A maximum acetic acid concentration reached up to 0.98 g/L after the 10^{th} day with a production rate of 0.098 g/L/d.

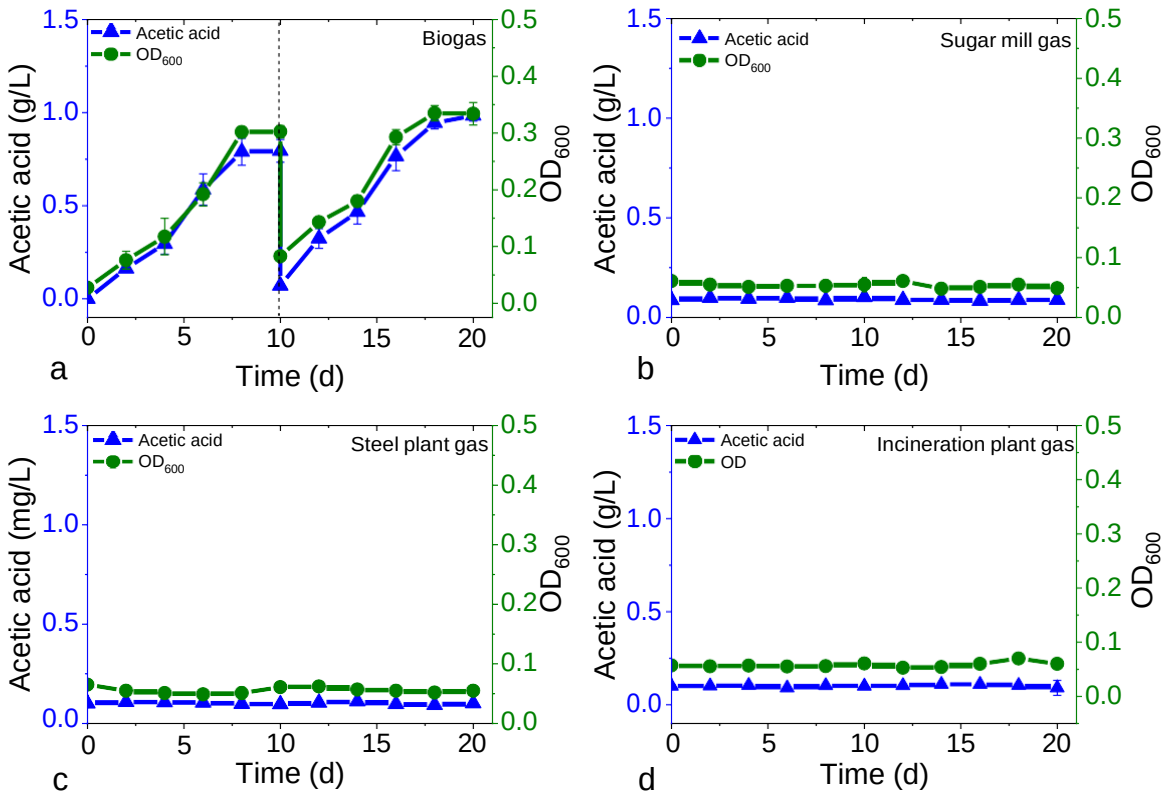

Figure 2.4: Microbial growth and acetic acid production profiles of the enriched mixed culture with different industrial CO_2 sources: a) Biogas b) Sugar mill gas, c) Steel plant gas, and d) Incineration gas sample.

However, no growth or organics production was detected with the incineration plant, sugar mill, and steel industry CO_2 sources (Figure 2.4 b-d). Despite of conducting gas fermentation experiments for longer batch cycles (~20 days) neither microbial growth nor any organics production was observed in these cases. It can be due to the presence of O_2 and traces of NOx and SOx in these industrial gas samples. Since the mixed microbial culture was unable to use this gas effluent in spite of the presence of some facultative microbes, this gas effluent was not tested with the pure culture, which is a strict anaerobe. Pretreatment of these industrial gases to remove toxic compounds might be beneficial for enhancing microbial tolerance to these CO_2 sources or avoiding the impact of impurities on microbial growth.

Neither organics production nor any increase in OD was observed in the biotic and abiotic control reactors (Figure 2.5). Hence, acetic acid production can exclusively be attributed to the microbial CO_2 fixation process with H_2 as the sole energy source in

the main experiments. The constant small amount of acetic acid was due to the residual components present in the inoculum.

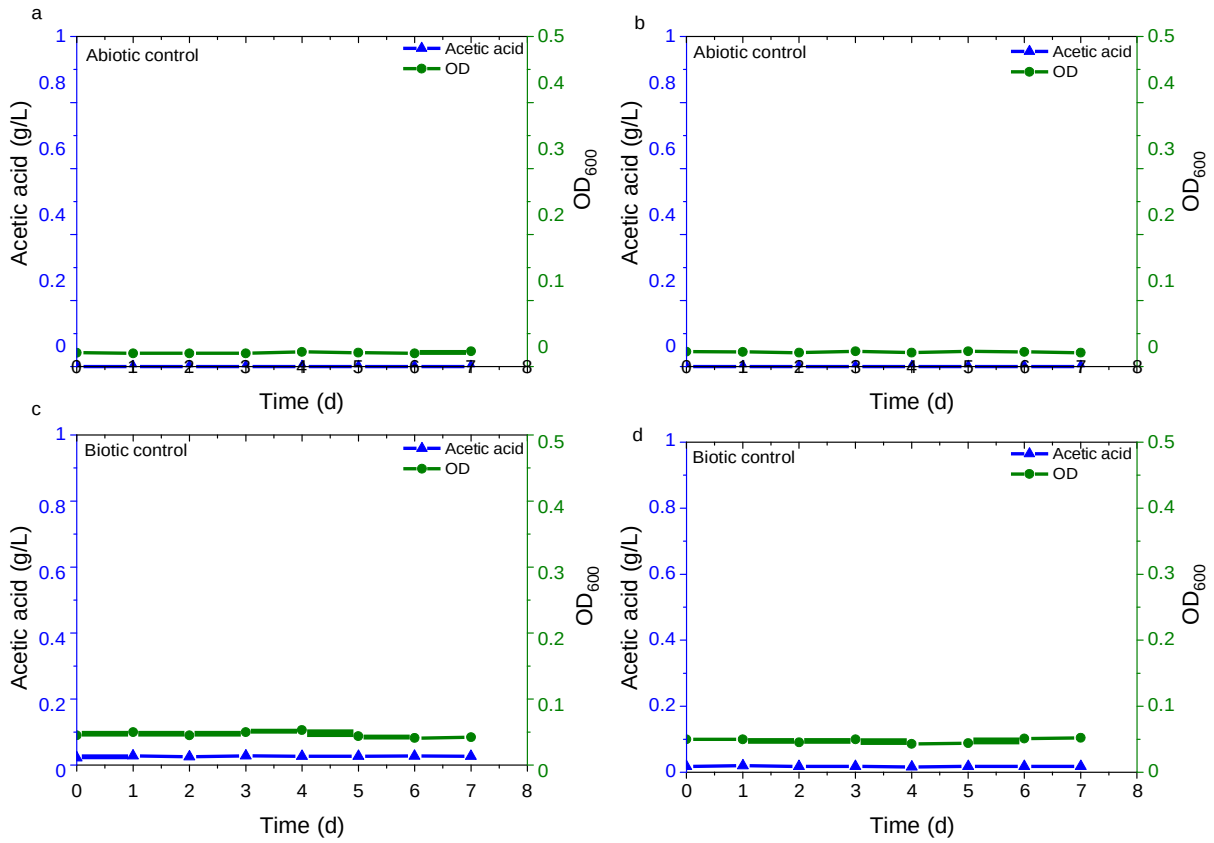

Figure 2.5: Microbial growth and acetic acid production profiles of the abiotic and biotic control experiments with the enriched mixed (a and c) and *Clostridium ljungdahlii* cultures (b and d).

2.4 Conclusions

Five different industrial gas samples were tested without any purification for bioproduction in gas fermentation experiments. Efficient acetic acid production and microbial growth were observed in the case of brewery CO_2 and biogas. Among the microbial cultures, *C. ljungdahlii* was more susceptible to the impurities in the industrial CO_2 than the mixed culture. Due to its robust and synergetic nature, the enriched mixed culture outperformed the pure culture. The gas samples that contained a high concentration of O_2 (e.g., incineration plant, sugar mill, and steel industry) could not be used without purification since O_2 is detrimental to the growth of anaerobic microbial cultures. The biogenic industrial sources like brewery CO_2 and biogas contained low impurities and O_2 that showed no significant detrimental effect

on microbial growth. Hence, further work was conducted with brewery CO_2 (chapter 3) and biogas (chapter 4).

Chapter 3

Assessment of microbial electrosynthesis technology for acetic acid production with brewery CO_2

This chapter focuses on the feasibility of utilizing unpurified brewery CO_2 through microbial electrosynthesis technology, followed by process optimization in an improved reactor through the following two major activities.

3.1 Unpurified brewery CO_2 utilization through microbial electrosynthesis for the production of acetic acid

3.2 Brewery CO_2 conversion into acetic acid at an optimized set of microbial electrosynthesis process parameters

Chapter 3.1: Unpurified brewery CO_2 utilization through microbial electrosynthesis for the production of acetic acid *

*The contents of this chapter are adapted with permission from the following peer-reviewed journal publication:

Roy, M., Yadav, R., Chiranjeevi, P., and Patil, S. A. Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis. Bioresource Technology. 2021, 320, 124289. 10.1016/j.biortech. 2020. 124289.

3.1.1 Introduction

The release of anthropogenic CO_2 has been on a continuous rise since the start of the industrial era. It has reached 33 gigatons by the end of 2019 (O'Neill, 2020) and is proving to be an enormous peril for the earth's climate as well as the ecosystem. The primary point sources of CO_2 emissions include cement and steel manufacturing industries, breweries, coal-powered power plants, and oil and gas production plants. The only enthralling part of these industrial emissions is that if the CO_2 is captured and appropriately utilized, it can be a valuable carbon source (Otto et al., 2015).

Several CCU technologies have emerged over the last two decades. The non-biological catalytic, photochemical, and electrochemical technologies are at different development stages to produce mostly single carbon-containing compounds (Martens et al., 2017). Biological processes such as algal cultivation, gas fermentation, and microbial electrosynthesis (MES) have emerged as prominent alternatives for producing multi-carbon chemicals (Kondaveeti et al., 2020). MES is primarily based on the use of microorganisms that possess the Wood- Ljungdahl pathway for fixing CO_2 (Ragsdale, 2008), and are relatively recent developments in the field of CCU. It is an electricity-driven bioproduction process, which offers the most direct way of supplying energy to the microorganisms *via* electrodes (Rabaey and Rozendal, 2010, Lovley and Nevin, 2013).

Since the publication of the first proof-of-concept MES study by Nevin et al. (2010), substantial advancements have been made in terms of efficient electrode materials and designs (Bajracharya et al., 2015, Chen et al., 2016, Aryal et al., 2017, Dong et al., 2018), the applicability of mixed and pure microbial catalysts (Patil et al., 2015a, Bajracharya et al., 2015, Bian et al., 2020), reactor design (Giddings et al., 2015, Bajracharya et al., 2016), the influence and optimization of operational conditions like electrode porosity (Jourdin et al., 2016), pH (Labelle et al., 2014), continuous flow mode and hydraulic retention time (Arends et al., 2017), cathodic potential (Mohanakrishna et al., 2016), media composition (Song et al., 2019), and the spacing between electrodes (Song et al., 2019) besides understanding the influence of interruption in electricity supply (Rojas et al., 2018a, 2018b) and developing downstream processes (Gildemyn et al., 2015) over the last decade. Though acetate has been the main target product of MES, the production of other chemicals such as

butyrate, caproate, butanol, and hexanol has also been reported (Blasco-G´omez et al., 2019, Das et al., 2020, Ganigué et al., 2015, Jourdin et al., 2018, 2019, LaBelle et al., 2020). Researchers have tried interlinking MES with other bioprocesses to produce longer-chain chemicals like polyhydroxybutyrates and wax esters (Sciarria et al., 2018, Lehtinen et al., 2017). A critical assessment of overall progress, practical applicability, scale-up challenges, techno-economic, and life cycle-related aspects of MES has been done recently by some researchers (Bian et al., 2020, Das et al., 2020, Jourdin et al., 2020, Prévoteau et al., 2020). It should be stressed here that the MES research is conducted with either bicarbonate or pure CO_2 as the feedstock. The feasibility of utilizing CO_2-containing industrial emissions via such carbon conversion processes thus remains unclear. This aspect has been pointed out (Bajracharya et al., 2015, Jourdin et al., 2020, Das et al., 2020); however, it remains to be investigated before this study. Considering the above-mentioned technological knowledge gap, this study aimed to investigate the influence of unpurified industrial CO_2 on the MES process. To this end, the brewery was chosen as an industrial source since it emits CO_2 with low impurities (Crabtree, 2017). Although it is not the major source of anthropogenic CO_2 emissions, it is a significant contributor. For instance, in the ethanol fermentation process from different feedstocks, about 0.32 to 5.55 kg CO_2 per liter of ethanol is produced in different geographical regions (Pacheco and Silva, 2019). If the worldwide production of 137.85 billion litres of ethanol is considered (https://www.grandviewresearch.com/industry-analysis/ethanol-market), a considerable amount of CO_2 is emitted by these industries. It makes breweries an attractive point source of CO_2 with minimum impurities for the production of chemicals. To better understand the influence of unpurified CO_2 on microbial activity and production process, two different microbial cultures, namely, an enriched mixed culture dominated by *Acetobacterium* sp. and a commonly used CO_2-fixing pure culture of *C. ljungdahlii,* were used. The biocathodes were subjected to electrochemical and microscopic analysis to understand the microbial role in the bioproduction process.

3.1.2 Methodology

All microbial and bioelectrochemical experiments were conducted at a controlled temperature of 28 ± 2 °C. If not stated otherwise, the electrode potential data are reported against Ag/AgCl (3.5 M KCl) reference electrode (0.205 V *vs.* SHE).

3.1.2.1 Microbial inoculum sources and cultivation conditions

Two different microbial inoculum sources, namely, an enriched mixed chemolithoautotrophic culture and a pure culture of *C. ljungdahlii*, were tested as biocatalysts. For the enriched mixed culture, anaerobic sludge from the municipal wastewater treatment plant (Mohali, India) was used as the inoculum source for enriching a chemolithoautotrophic culture (section 2.2.3.1). The enriched culture's microbial community composition was based on the 16S rRNA sequencing-based analysis (section 3.1.2.6). *Acetobacterium* (26 %), *Pseudomonas* (12 %), and *Sulfurospirillum* (24 %) were found to be the most dominant genera in the enriched culture (section 3.1.3.5). The pure culture of *C. ljungdahlii* DSM13528 was obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). It is a gram-positive, rod-shaped obligate anaerobe and non-pathogenic bacteria. It can utilize H_2 as an energy source to fix CO_2 *via* the Wood-Ljungdahl pathway and is a commonly used microbial catalyst in MES experiments (Nevin et al., 2011, Bajracharya et al., 2015). Hence, it was chosen as a representative pure microbial strain in this study. It was cultivated according to DSMZ medium 879.

3.1.2.2 Industrial gas sampling

The gas samples were collected from a brewery industry (Om sons Marketing Pvt. Ltd, Bathinda; ferment barley grains) in 6 L passivated SilcoCan steel canisters (Restek, USA) using the same set up as mentioned in 2.2.4. The brewery gas emissions contained mainly CO_2 (97.91 ± 1.52 %), N_2 (1.72 ± 0.70 %), and O_2 (0.356 ± 0.1 %) (Note: 1 % = 10000 ppm). The detection of a trace amount of O_2 can be attributed to manual errors during either sampling or sample injection in the GC system. Also, traces of hydrocarbons (~ 130 ppb) were detected in the brewery gas. These include mainly alkanes (e.g., butane, pentane, octane, and heptane), alkenes

(e.g., ethene, isoprene, butane, and propene), and aromatics (e.g., benzenes, toluene, ethylbenzene, and xylene). Other components in the brewery gas were acetaldehyde (44 ppm), ethanol (16 ppm), and methanol (0.4 ppm).

3.1.2.3 MES reactor setup and experiments with industrial CO_2

MES experiments were conducted in the two-chambered customized glass-made bioelectrochemical reactors under potentiostatically-controlled conditions (VMP3, BioLogic Science Instruments, France). Electrochemically, these were operated in a three-electrode configuration mode. A graphite plate of 10 cm^2 projected surface area served as the working electrode (i.e., cathode). Before use, it was pretreated by the acid-alkali method to remove impurities and polished using alumina powder. Dimensionally stable mixed metal oxide coated titanium plate with a 7.5 cm^2 projected surface area was used as the counter electrode (i.e., anode). The reference electrode was Ag/AgCl (3.5 M KCl, + 0.205 V *vs*. SHE, RE-1B, BioLogic Science Instruments, France) and placed in the cathode chamber. Both the anode and cathode electrodes were separated by a 117 Nafion proton exchange membrane (Sigma Aldrich). Before use, the Nafion membrane was pretreated at 70–80 °C sequentially in different solutions as follows: deionized water for 1 h, 2 % H_2O_2 for 1 h, rinsing with normal deionized water, 4.9 % H_2SO_4 for 1 h, and rinsing in deionized water. Each electrode chamber had a 350 mL empty bed volume and three ports for electrode placement and sampling purposes. The working volume of the anolyte and catholyte was 250 mL. The anolyte was 0.5 M Na_2SO_4 with pH 2.5 (adjusted with 1 M H_2SO_4). The catholyte was the same media as discussed in gas fermentation experiments. Every day 20 mL of CO_2 containing brewery gas was added to the reactors, which served as the only carbon source. A cathodic potential of − 1.0 V and − 1.2 V (*vs.* Ag/AgCl) was applied in the reactors with enriched mixed culture and *C. ljungdahlii*, respectively, and the reduction current response was monitored at a set time interval of 5 min by using chronoamperometry (CA) technique. The composition and pH of the growth cum electrolyte media used for the enriched mixed and pure cultures were different. According to the cyclic voltammetry data of the abiotic cathode, the hydrogen evolution reaction occurred at a lower potential of ~ − 1.0 V *vs*. Ag/AgCl in the growth medium with pH 6 used for the *C. ljungdahlii* culture compared to ~ − 0.840 V in the case of mixed culture medium with pH 7. It is in agreement with the

Nernst equation, according to which the pH affects the electric or redox potential values. Hence, based on the cyclic voltammetry observations and to ensure a continuous supply of H_2 to microorganisms, different and slightly lower applied potentials were chosen in MES experiments with mixed and pure cultures. The MES experiments were conducted in three batch cycles by replenishing the spent medium at the end of each cycle. The gas and liquid samples were taken every day for further analysis. Two control experiments, namely biotic-electrochemically unconnected (inoculated but not connected electrochemically, thus no electron source) and abiotic-electrochemically connected (electrochemically connected but uninoculated, thus only abiotic or electrochemical catalysis), were conducted to confirm the microbially-catalysed electricity-driven bioproduction. Cyclic voltammograms (CVs) were recorded in a potential window of − 1.4 and − 0.2 V at a scan rate of 1 mV s^{-1} at different conditions, namely, before inoculation, after inoculation, and at the beginning and end of each batch cycle.

3.1.2.4 Analysis and calculations

The liquid samples from both the gas fermentation and MES experiments were analysed for organics production (C1-C4 organic acids and alcohols) by HPLC (Agilent 1260 Infinity II, RID Detector, Hiplex H column, 5 μM H_2SO_4 mobile phase, flow rate 0.5 mL/min, temperature 50 °C), microbial growth by monitoring OD at 600 nm using UV–VIS spectrophotometer (Photo-lab 7600), and pH (Oakton PC2700). The biomass produced in MES experiments was estimated based on insoluble COD (Rice et al., 2012). The gas samples from the headspace of serum flasks and MES reactors were analysed for H_2, CO_2, O_2, CH_4, and H_2S 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]. For MES, product titer, production rates (volumetric and cathode surface area-based), and coulombic efficiency (electron recovery into products) were calculated according to protocols described elsewhere (Patil et al., 2015b). The data from the batch cycles in which the maximum acetic acid concentration was achieved in duplicate reactors were considered. H_2 consumption rate was not calculated since the amount of dissolve H_2 was not measured in case of this study.

3.1.2.5 Scanning electron microscopy (SEM) of the biocathode and the bulk phase samples from MES reactors

The biocathodes (i.e., cathode with the microbial biofilm) were cut in a 1 cm length size. These, along with the bulk phase samples, were fixed with 2 % glutaraldehyde and 2.5 % paraformaldehyde solution at 4 °C overnight. These samples were incubated in 1 % osmium tetraoxide for 90 min for post-fixation, followed by dehydration in different dilutions of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, and 100 %). Then, the samples were dried in a silica desiccator. Finally, they were sputtered with gold particles by JEOL JEC-1600 Auto-Fine Coater (JEOL Ltd., Japan) at 20 mA for 45 s and analysed with the JEOL JSM-6010PLUS/LS microscope (JEOL Ltd., Japan).

3.1.2.6 Microbial community analysis in the enriched mixed culture-based MES experiments

DNA was extracted from the inoculum source used for enriching the chemolithoautotrophic culture (i.e., anaerobic sludge), enriched mixed culture, along with the cathodic biofilm and bulk phase of the MES reactor using QIAGEN DNeasy PowerSoil Pro Kit. The DNA samples were quantified by Nanodrop (Genova Nano – 4359, Jenway, Cole- Parmer, UK), and Qubit fluorimeter (V.3.0, Thermo Fischer Scientific, USA). Agarose gel electrophoresis was performed to check the integrity of samples. To synthesize 16S rRNA sequences, the isolated DNA samples were used as templates. The V3-V4 regions of 16S rRNA sequences were amplified with specific V3 Forward primer 5′ - GCCTACGGGNGGCWGCAG-3′ and V4 Reverse primer 5′ - ACTACHVGGGTATCTAATCC-3′. The amplified products were analysed by 2 % agarose gel electrophoresis. The 16S rRNA amplicon sequencing was performed at the Miseq 2500 platform at Eurofins Genomics India Pvt. Ltd (Bangalore, India). The high-quality reads were trimmed using Trimometic V0.38. FLASH V 1.2.11 was used to align forward and reverse reads, and the operational taxonomic unit (OTU) picking was performed by the same tool. Finally, the resultant OTUs were aligned with the Silva database using the Qiime1 pipeline, and R packages were used to visualize the results. The raw sequencing data files are available on the NCBI short-read archive under the SRR12539094-97 accession numbers.

3.1.3 Results and Discussion

3.1.3.1 MES from unpurified industrial CO_2

Both cultures utilized industrial CO_2 for growth and organics production in MES experiments (Figure 3.1.1). In these experiments as well, the major organics produced was acetic acid. Immediate start-up in acetic acid production and a clear correlation between organics production and growth was observed (Figure. 3.1.1). For enriched mixed culture, acetic acid production levelled off on or after the seventh day in each batch cycle (Figure. 3.1.1a). A maximum acetic acid titer of 1.8 ± 0.2 g/L was achieved on the seventh day of the third batch cycle. It is similar to previously reported studies with pure CO_2 feed (Mateos et al., 2019). It was produced at a volumetric rate of 0.26 ± 0.03 g/L/d, and a cathode surface area-based rate of 66 ± 5.7 $g/m^2/d$. Formic (0.25 ± 0.007 g/L) were also produced regularly, but in low concentrations by the mixed culture during the initial stages. Formic acid serves as a crucial intermediate in the Wood-Ljungdahl pathway (Fig. 1.3) by facilitating the conversion of carbon dioxide into acetyl-CoA, which further gets metabolized to produce acetic acid via this pathway. Apart from that, butyric acid (0.12 ± 0.017 g/L) was also detected. The carbon recovery efficiency was around 84% with the brewery flue gas. About 84 ± 13 %, 5 ± 1.2 %, 3.5 ± 3 %, and 2 ± 1.5 % electrons were recovered in acetic acid, other organics, H_2, and biomass analysed from the bulk phase. Since the major focus of this study was on production utilizing industrial CO_2-rich gas, the biomass was estimated only at the start and end of the cycle.

Methane was not detected in the MES reactors, thereby suggesting successful inhibition of methanogens in the enriched culture.

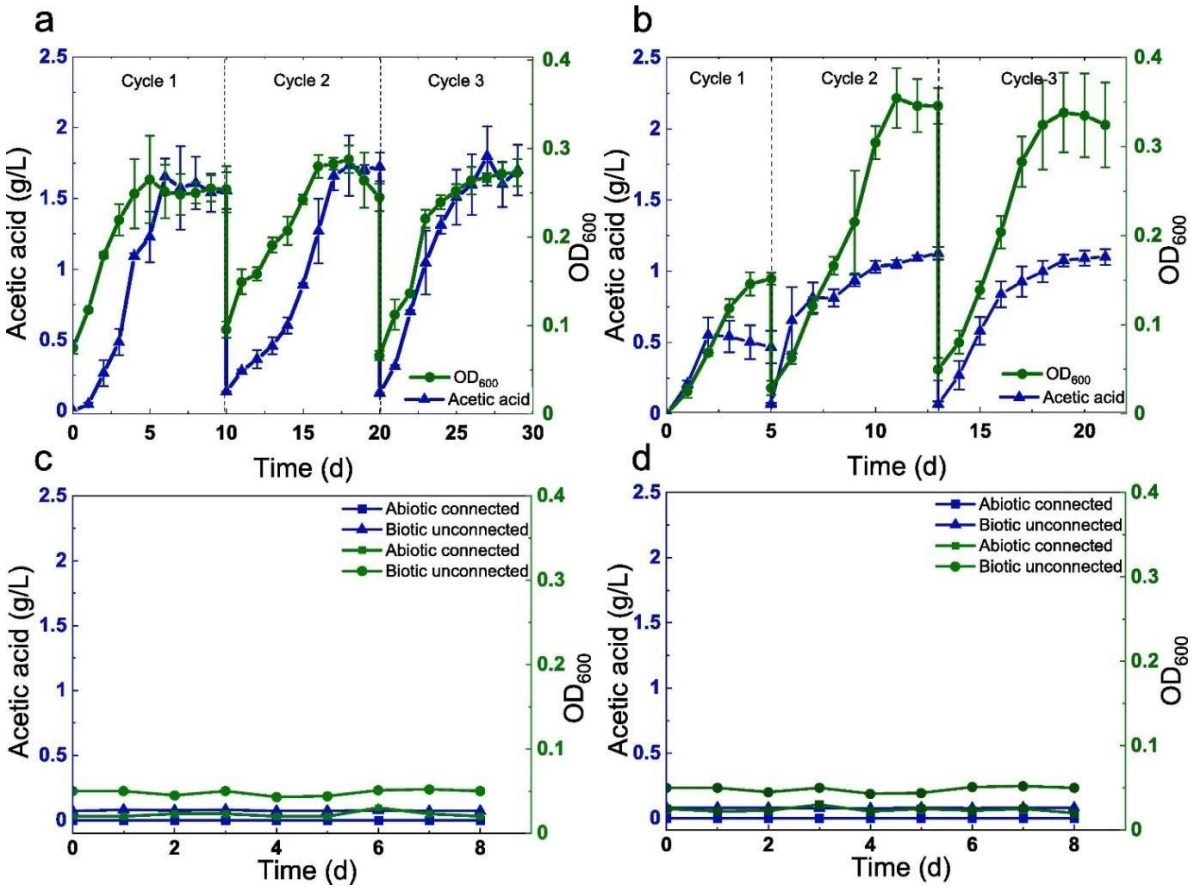

Figure 3.1.1: Acetic acid production and growth (as OD_{600}) profiles of the enriched mixed (a) and *C. ljungdahlii* (b) cultures fed with unpurified industrial CO_2 in MES experiments. The dotted line in a and b indicates the start of the new batch cycle. The bottom panel shows the data of abiotic-electrically connected and biotic-electrically unconnected control experiments for the enriched mixed (c) and *C. ljungdahlii* (d) cultures.

For *C. ljungdahlii*, acetic acid production was lower in the first batch cycle than in subsequent batch cycles (Figure 3.1.1b). In the second cycle, the acetic acid titer increased considerably, and during the third batch cycle, it remained in a similar range. A maximum acetic acid titer of 1.1 ± 0.02 g/L was achieved at the volumetric and cathode surface area-based production rates of 0.138 ± 0.004 g/L/d and 34.6 ± 1.1 $g/m^2/d$, respectively by *C. ljungdahlii*. It also produced formic acid up to 0.24 ± 0.013 g/L. About 42 ± 14 %, 4 ± 1 %, 17 ± 2 %, and 6 ± 2 % of electrons were recovered in acetic acid, formic acid, H_2, and suspended biomass, respectively, in this case. The unaccounted electrons can be attributed to the cathodic biomass and non-analysed organic products in both mixed and pure culture reactors. The hydrogen loss was higher in the case of the pure culture (around 30%) compared to that of the enriched

mixed culture (around 20%). Besides limited utilization of the produced H_2, this was majorly due to the lower applied cathode potential and higher current draw in the case of the *C. ljungdahlii* culture. The pH also remained in a similar range with both inoculum sources, and no alcohol production was observed in any of the MES experiments. No increase in the OD and organics production was observed in biotic-unconnected and abiotic-connected control experiments conducted along with the main MES experiments (Figure 3.1.1c and d). These observations confirm the electricity-driven production of organic acids from unpurified industrial CO_2 by the microbial activity in MES tests. The MES experiments also revealed that the mixed culture outperforms the pure culture in organics production from industrial CO_2. The potential at the anode (i.e., E_{anode}) was 1.8 ± 0.17 V and 2.3 ± 0.4 V for mixed culture and *C. ljungdahlii* reactors, respectively. Considering the applied $E_{cathode}$ of − 1.0 V and − 1.2 V for the mixed culture and *C. ljungdahlii* reactors, the production occurred at the respective E_{cell} of 2.8 V and 3.5 V. These values are in the similar range reported in the earlier studies that have been conducted at comparable experimental conditions (Bajracharya et al., 2015, Song et al., 2017, Mateos et al., 2019).

3.1.3.2 Electrochemical analysis of biocathodes suggests the role of microorganisms in improved electrocatalysis

The CVs recorded during the control conditions, i.e., before and immediately after inoculation, revealed no redox peaks and, therefore, no redox-active moieties at the cathode surface and in the catholyte of MES reactors (Figure 3.1.2). In a mixed culture reactor, the H_2 evolution potential was ~ − 0.840 V at the abiotic cathode (Figure 3.1.2a). An apparent shift in H_2 evolution potential to around − 0.6 V was observed in the biocathode CV recorded at the end of MES experiments. It suggests a considerable lowering of the H_2 evolution overpotential (by > − 0.200 V) at the biocathode compared to the abiotic cathode.

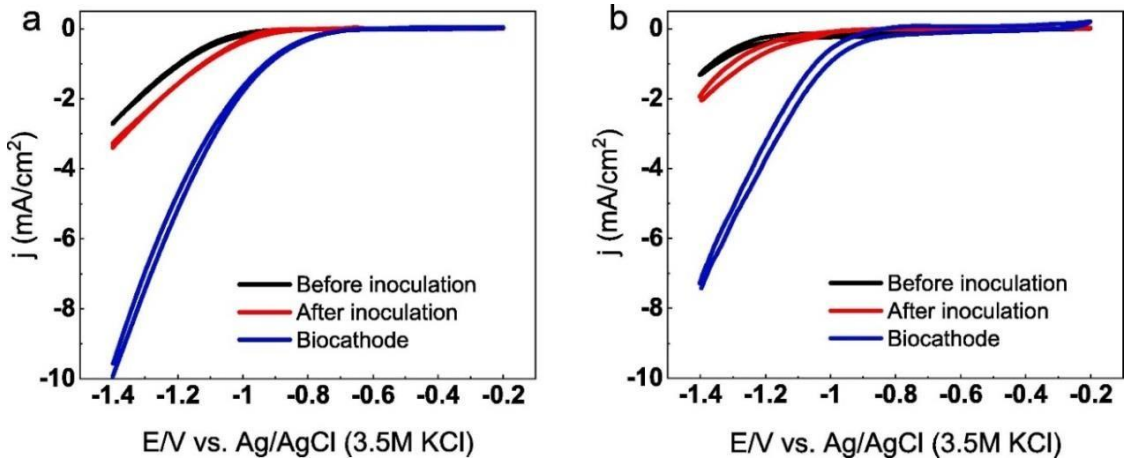

Figure 3.1.2: Representative cyclic voltammograms recorded at different conditions with the enriched mixed culture (a), and *C. ljungdahlii* (b) inoculated MES reactors (scan rate: 1 mV/s).

Similar behaviour of lowered H_2 evolution overpotential at the biocathode compared to the abiotic cathode was observed in the CVs recorded for *C. ljungdahlii* reactors (Figure 3.1.2b). From the CV recorded before inoculation (i.e., abiotic cathode), the H_2 evolution potential was found to be around − 1 V. A lower potential in this case than the mixed culture inoculated reactor was due to the low pH medium used as the catholyte.

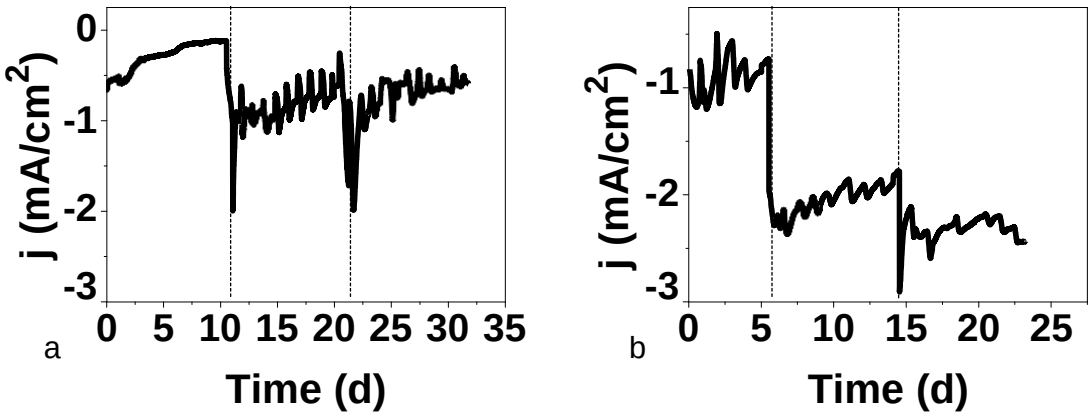

Figure 3.1.3: Representative chronoamperometry profiles of the MES reactors inoculated with the enriched mixed culture (a) and the *C. ljungdahlii* culture (b).

Moreover, the cathodic current draw also increased greatly at the biocathode (e.g., − 1.25 and – 2.5 mA/cm^2 for the enriched mixed and *C ljungdahlii* cultures, respectively), suggesting enhancement in electrocatalysis at the cathode (Figure 3.1.3). The improved electrocatalytic performance can be attributed to microbial

activity. It is prominently visible that during the first cycle, the reduction current was quite low. In subsequent cycles, the reduction current increased significantly, approximately double that of the first cycle. This suggests a progressive improvement of the electrocatalysis process over successive fed-batch cycles.

The SEM analysis confirmed the growth of microorganisms at the cathode surface and in the bulk phase of reactors. For enriched mixed culture, full coverage of the cathode surface by microbial biofilm was observed. The uniform biofilm growth correlated with and supported the improved electrocatalysis observed at the cathode in this case (Figure 3.1.4 a, b). However, for *C. ljungdahlii*, less growth at the cathode surface and more in the bulk phase was observed (Figure 3.1.4 d, e). These observations are in agreement with the OD data of the MES reactors (Figure 3.1.1).

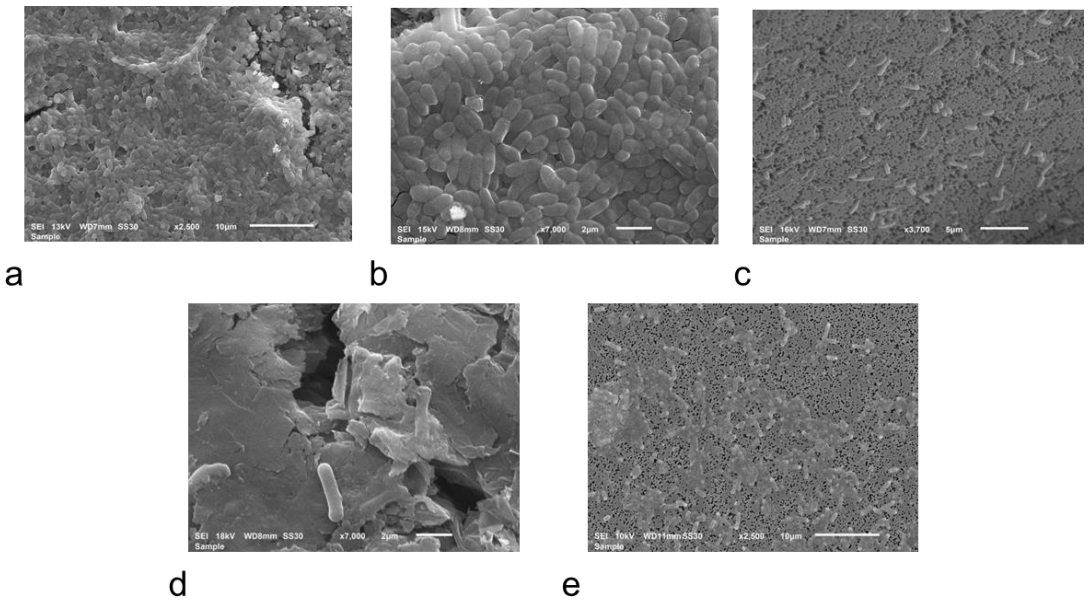

Figure 3.1.4: Representative SEM images of the enriched mixed culture (Biocathode: a, b, Bulk phase: c), and *C. ljungdahlii* (Biocathode: d, Bulk phase: e) inoculated MES reactors.

3.1.3.3 Microbial community analysis of the enriched mixed culture

Acetobacterium, Pseudomonas, Desulfovibrio, Sulfurospirillum, Proteiniphilum, and Bacteroides genera dominated the enriched mixed culture at relative abundances of 26.4 %, 12.6 %, 4.5 %, 24 %, 6.5 %, and 7.4 %, respectively (Figure 3.1.5-EC). The same enriched microbial culture was used in the MES reactor. Both biofilm-driven and H_2-mediated electron transfer might have occurred in the reactors. The H_2-driven (bulk phase) production was more prominent. Therefore, solution-based (bulk phase-

based) screening method was useful for MES application.Cathodic biofilm-based screening methods for different gases can be another strategy, but in that case, the yield might be limited compared to the bulk phase-based screening approach.

In MES reactors, *Acetobacterium* dominated both the biocathode and suspension community at relative abundances of 10.6 % and 11 %, respectively (Figure 3.1.5-MES-biocathode and MES-suspension). It was present only at 3.7 % relative abundance in the original anaerobic sludge inoculum source used to enrich the CO_2 fixing culture (Figure 3.1.5-AS). *Acetobacterium* group is a well-known acetogen that fixes CO_2 using H_2 to grow and produce acetic acid as the primary metabolite *via* the Wood-Ljungdahl pathway (Ragsdale, 2008). In addition to acetate and formate, some *Acetobacterium* spp. can produce butyrate (Labelle et al., 2020). It aligns well with the bioproduction data of both serum flask and MES experiments (sections 2.3.2.1 and 3.1.3.1) in this study. *Desulfovibrio* was one of the dominant genera in MES reactors at up to 9 % relative abundance. They are the major sulfate-reducing microbes, which most likely were enriched due to the sulfate-containing medium. They can uptake electrons directly from a cathode to reduce H^+ ions to H_2 gas (Aulenta et al., 2012), and have been reported in MES reactors earlier (Labelle et al., 2014, Patil et al., 2015a, Song et al., 2017, Mateos et al., 2019). Thus, they can be implicated in H_2 production in the MES reactors. Some *Desulfovibrio* spp. are also capable of producing formate through the activity of cytochromes, hydrogenases, and formate dehydrogenase (Labelle et al., 2020). Both H_2 and formate are energy carriers that can be used by acetogenic bacteria present in the cathodic biofilm and the bulk phase of MES reactors. Two other microbial genera, namely *Pseudomonas* and *Sulfurospirillum,* were present at the respective relative abundances of up to 26 % and 13 % in MES reactors. *Pseudomonas* spp. have been reported in previous MES studies (Rojas et al., 2018a, Mateos et al., 2019). They can play an important role in the electron transfer process through hydrogenase enzymes and electron mediators (Mateos et al., 2019). *Pseudomonas* is also known to metabolize a wide range of aliphatic and aromatic hydrocarbons and their derivatives. The hydrocarbons present in the brewery gas might have been used as carbon and electron sources by these microbes. *Sulfurospirillum* genus is microaerophilic and can scavenge trace amounts of O_2 and help maintain anaerobic conditions in the MES reactors (Goris et al., 2014). It can also oxidize acetic acid using O_2 as an electron acceptor.

Hence, it can be deduced that their presence can decrease the acetic acid titers marginally. However, they can play a crucial role in scavenging O_2 traces present in the unpurified industrial CO_2, and that can crossover from the anode to the cathode chamber in bioelectrochemical systems, thereby maintaining the overall productivity of the MES process. Other microbial groups that were dominant in the MES reactor include *Bacteroides* (9 %), *Proteiniphilum* (8 %), *Sphaerochaeta* (6 %). Without further understanding, attributing any role to these microbes in the MES process would be speculative.

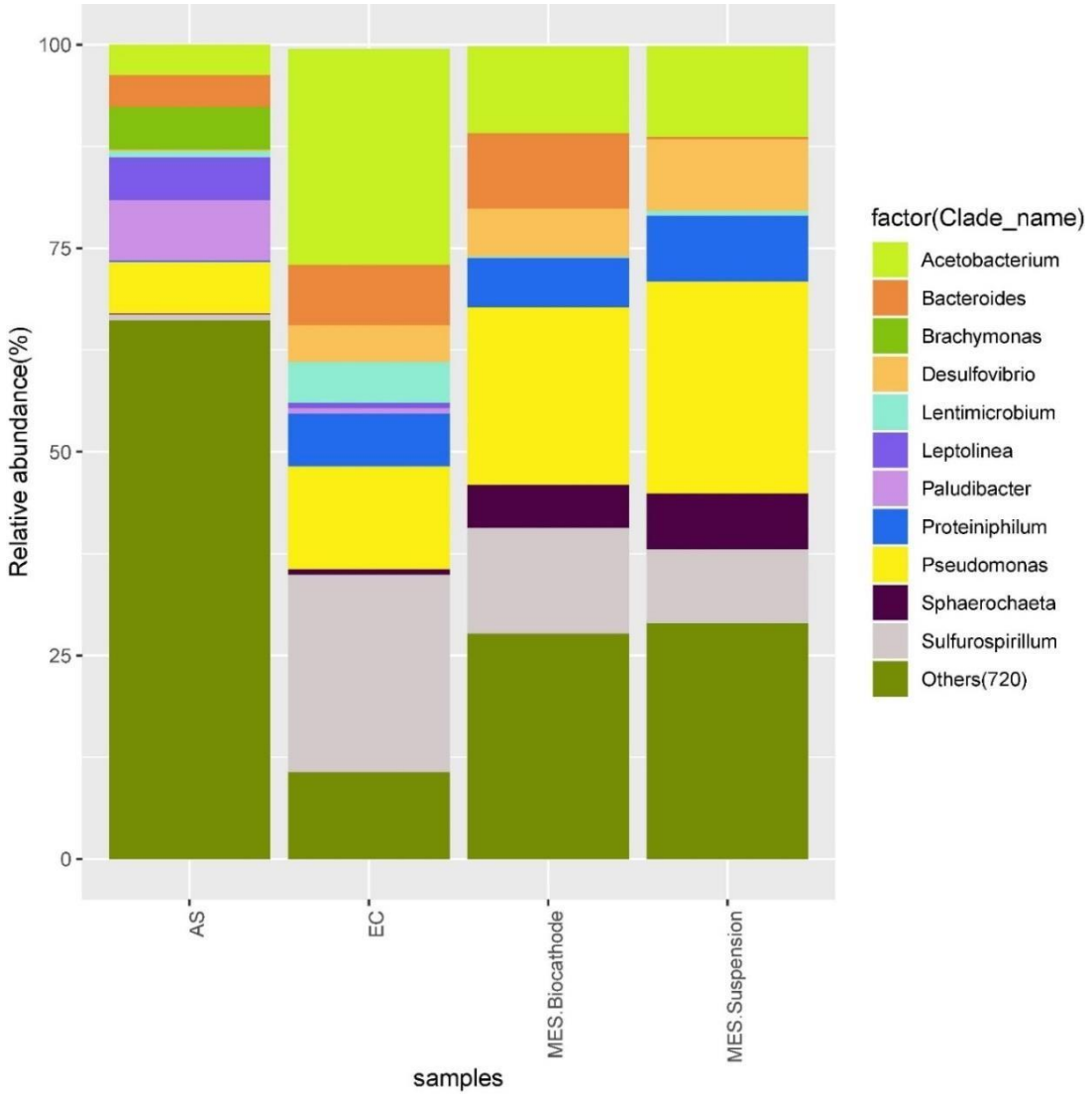

Figure 3.1.5: 16S rRNA sequencing-based microbial community composition at the genus level in the activated sludge (AS) inoculum source used for the enrichment of CO_2 fixing culture, enriched CO_2 fixing mixed culture (EC) used in microbial electrosynthesis (MES) experiments, along with the MES-biocathode and MES-suspension samples.

3.1.3.4 Pure vs. enriched mixed cultures for the utilization of unpurified industrial CO_2

Based on the key production parameters like production titer, rate, and coulombic efficiency, it is evident that enriched mixed culture outperformed *C. ljungdahlii* for utilizing unpurified industrial CO_2 in the MES experiments (Figure 3.1.6). It can be attributed to the robustness and diverse functionality of the mixed microbial community, as discussed in the previous section. Different microbial groups most likely help the mixed community deal with different impurities present in the industrial gas. For example, *Sulfurospirillum* can consume O_2 and avoid its accumulation in the system. Another dominant group *Pseudomonas*, has hydrocarbon-degrading capabilities (Timmis, 2010). This particular metabolic feature is useful in dealing with the traces of hydrocarbons present in industrial gas. The syntrophic and mutualistic relationships among mixed communities can also enhance the overall performance of the system. In the case of a pure culture, all these possibilities lacked, and thus, their growth and performance were adversely affected. The use of mixed microbial communities also offers a major advantage over the emerging electrochemical or catalytic processes for CO_2 utilization because the abiotic catalysts can easily get affected or poisoned by the low concentrations of impurities present in industrial gases. A key issue with mixed microbial communities is the production of a mixture of organics, which can be circumvented by maintaining controlled medium and operational conditions. The microbial population in mixed cultures is also challenging to control and is prone to changes in response to drastic changes in operational conditions. However, if the operational conditions are maintained or restored to the original set of conditions, mixed cultures‘ performance can be restored to the earlier level. It should also be noted that mixed cultures are robust; thus, any slight or minor changes in the operational conditions do not easily affect their performance or desired functionality. These observations have been noted earlier in MES studies conducted over extended periods of several months to more than a year (Arends et al. 2017, Bajracharya et al., 2017a, Jourdin et al. 2018).

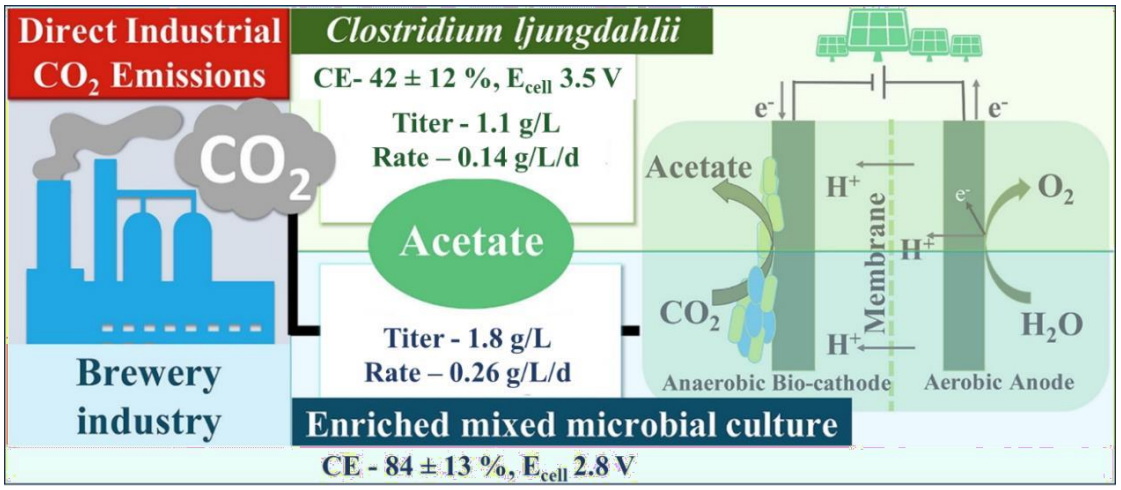

Figure 3.1.6: Comparison of the pure and enriched mixed microbial cultures for brewery CO_2 utilization in MES systems.

3.1.3.5 Performance of MES with unpurified industrial CO_2 vs. pure CO_2 or bicarbonate

MES experiments have been conducted mostly with bicarbonate as the carbon source using both pure and mixed microbial cultures so far. In the present study with unpurified industrial CO_2, a maximum acetic acid titer of 1.8 g/L was achieved at a production rate of 0.26 g/L/d with 84 % electron recovery by the enriched mixed culture. These values are comparable with the previous MES studies conducted with pure CO_2 or bicarbonate feeds under a similar set of experimental conditions. For instance, with bicarbonate feed, acetic acid titer in the range of 0.63 - 4.05 g/L with 15 - 70 % electron recovery has been reported (Rojas et al., 2018b, Mohanakrishna et al., 2016, Bajracharya et al., 2015). In the case of pure CO_2, 4.8 - 5.45 g/L acetate production, along with 70 - 85 % electron recovery, has been achieved with the mixed microbial cultures (Song et al., 2017, Mohanakrishna et al., 2016, Chu et al., 2020). Although with the pure culture of *C. ljungdahlii*, acetic acid production (1.1 g/L) from industrial CO_2 was lower in the present study, it is comparable to the reported values with pure CO_2 and bicarbonate feeds (Nevin et al., 2011, Bajracharya et al., 2015). Acetate production at high titers and rates has been reported with highly efficient and three-dimensional cathodes and at an optimized set of experimental conditions. For instance, a very high acetate production rate of 1330 $g/m^2/d$ and 99 % electron recovery has been reported with bicarbonate feed, and multiwalled carbon nanotubes-reticulated vitreous carbon as the cathode (Jourdin et al., 2016). At the optimized set

of operational conditions and efficient cathode material, a similar MES performance level can be expected with the unpurified brewery CO_2 feed. Further investigations are needed to check the MES performance with other industrial gases that contain more impurities. By using direct CO_2-containing industrial gases and avoiding costly purification steps, the process cost can be reduced considerably, which is a desirable step in the right direction to make MES feasible for implementation.

3.1.4. Conclusions

The key conclusion of this study is the successful demonstration of the direct use of unpurified industrial CO_2 for bioproduction *via* gas fermentation and MES with enriched mixed and pure microbial cultures. The mixed culture outperformed the pure culture for acetic acid production, most likely due to its robustness and diverse functionality in coping with industrial gas impurities. By reporting on the utilization of industrial CO_2 with low impurities, this study opens up the possibilities of using other flue gases for bioproduction and offers a major advantage over electrochemical or catalytic CO_2 conversion processes that can be adversely affected by even traces of impurities.

Chapter 3.2: Brewery CO_2 conversion into acetic acid at an optimized set of microbial electrosynthesis process parameters

The contents of this chapter are submitted to a peer-reviewed journal for publication:

- **Roy M.,** Kumar S., Patil SA. Brewery CO_2 conversion into acetic acid at an optimized set of microbial electrosynthesis process parameters. Journal of Environmental Chemical Engineering. 2024 (submitted)

3.2.1 Introduction

Industrial point sources, such as power plants, cement industries, breweries, steel plants, and refineries, release gaseous emissions with a high CO_2 concentration and play a significant role in contributing to global warming (Dessi et al., 2020, McLaughlin et al., 2023). While industrial point sources contribute significantly to greenhouse gas (GHG) emissions, they also offer the possibility of focused mitigation efforts involving capture and storage or utilization of CO_2 via different strategies. Efforts to mitigate carbon emissions focus on implementing carbon capture and utilization (CCU) technologies, improving energy efficiency, and transitioning to renewable energy sources to reduce the carbon footprint of industrial activities (Mikulčić et al., 2019, Roy et al., 2023). The atmospheric CO_2 is too diluted to be used as a carbon feedstock via various carbon capture and utilization (CCU) technologies, hence requiring prior concentration steps that incur huge costs. Extracting CO_2 directly from industrial point sources offers a promising avenue for obtaining a concentrated CO_2 stream at reduced transportation and purification expenses, thereby enhancing the feasibility of economically viable CCU technologies (Mikulčić et al., 2019). Nonetheless, the presence of contaminants like SOx and NOx, CO, fly ashes, and volatile organic compounds in flue gas necessitates substantial gas cleaning and conditioning before it can be utilized as feedstock through non-biological CCU methods (Pappijn et al., 2020).

In this context, bio-based CCU approaches like algae cultivation, gas fermentation and microbial electrosynthesis (MES) can be beneficial in utilizing the industrial CO_2-containing gases ‒as is‖ without any purification or cleaning. For instance, algae are proven to utilize different CO_2-rich industrial gases as a carbon source (Singh et al., 2016, Singh et al., 2019, Cheng et al., 2019, Cutshaw et al., 2020). For instance, Cutshaw et al. (2020) demonstrated the long-term utilization of power plant flue gas in a pilot-scale reactor. Yang et al., (2021) evaluated the techno-economic and environmental aspects of sulphur, urea and methanol production from flue gases. The possibility of CO_2 utilization and the importance of genetic modification and improvement of microalgal strains to withstand toxic impurities (such as SOx, NOx and fly ashes) present in coal-fired flue gas was also studied (Cheng et al., 2019, Aslam et al., 2017). In the case of the gas fermentation process, both pure as well as mixed microbial catalysts have been proven to work with industrial CO_2 from

different industries like the brewery and steel plants (Novak et al., 2021, Roy et al., 2021a). The process has been scaled up to an industrial level by LanzaTech, where unpurified industrial gases from the steel industry, refinery, and power plants are used to produce an array of value-added products. Unlike algae cultivation and gas fermentation, using unpurified industrial CO_2 via MES for bioproduction remains less explored.

MES, an electricity-driven bioproduction process from CO_2 using microbial catalysts in bioelectrochemical systems, has been tested for industrial CO_2 utilization recently. Recent studies have investigated the feasibility of producing various products through MES, including acetic acid, methane, and lycopene, utilizing real exhaust gases, such as those from breweries, power plants, and steel mills, as carbon sources in MES reactors (Roy et al., 2021a, Rovira-Alsina et al., 2022, Spiess et al., 2022, Wu et al., 2022). For instance, Rovira-Alsina et al., 2022 reported CO_2 reduction into acetic acid using an MES system with real off gas containing a significant amount of oxygen (12 %). However, challenges such as compromised methane production have been observed when utilizing steel industry off-gases (Spiess et al., 2022). Lycopene production up to 1.73 mg/L was reported with a coal-fired power plant exhaust gas in the MES system (Wu et al., 2022). It has been highlighted in the proof-of-concept MES study without any process optimization that the brewery CO_2 typically contains low levels of toxic impurities, making it a promising feedstock for MES processes (Roy et al., 2021a). As a logical follow-up to the previous work, this study aimed to assess the MES productivity from brewery CO_2 at different process operational parameters, including gas feed rate and applied cathode potential. Optimization of such parameters can lead to enhancing production efficiency and scalability, ultimately improving the technology readiness level of MES systems for industrial CO_2 utilization. Recirculation of liquid and CO_2 in the cathode chamber is commonly followed in MES studies to enhance production. The effect of gas recirculation has been studied earlier and is found to be beneficial for improving overall productivity (Mateos et al., 2019, Bajracharya et al., 2022, Pan et al., 2023). Though recirculation of catholyte is routinely employed in MES studies (Arends et al., 2017, Rovira-Alsina et al., 2022, Cui et al., 2023, Deutzmanne and Spormann, 2024), its actual impact of performance enhancement compared to MES reactors without catholyte recirculation has been barely studied. Since liquid recirculation incurs additional costs due to pump

use, it is imperative to understand how much productivity enhancement it actually leads to for the rational design and operation of scale-up systems. Overall, this study reports on the MES performance enhancement at optimized brewery CO_2 feed rate and applied $E_{cathode}$ and contributes to a better understanding of catholyte recirculation on the acetate productivity.

3.2.2 Methodology

3.2.2.1 Microbial inoculum source and growth medium

Previously reported *Acetobacterium*-dominated enriched mixed culture was used as microbial inoculum in the cathode chamber of the MES reactors (Roy et al., 2021a). It was enriched from anaerobic sludge from the municipal wastewater treatment plant (Mohali, India) under H_2:CO_2 conditions at 28 ± 2 °C in a minimal medium (with pH 7) containing K_2HPO_4 (5.35 g/L), KH_2PO_4 (2.62 g/L), NH_4Cl (0.25 g/L), KCl (0.5 g/L), $CaCl_2.2H_2O$ (0.15 g/L), $MgCl_2.2H_2O$ (0.6 g/L), L-cysteine (0.5 g/L), trace metal solution (10 mL), selenium – tungstate solution (1 mL), vitamin solution (20 mL), 2-bromoethanesulfonate (6.4 g/L), and $Na_2S.9H_2O$ (0.3 g/L), and 1 ml resazurin (stock: 1 g/L). The medium was boiled and cooled down under N_2 gas-sparging conditions in order to maintain anaerobic conditions before autoclaving.

3.2.2.2 Brewery gas source and composition

Gas samples from the brewery industry (Om Sons Marketing Pvt. Ltd, Bathinda; ferment barley grains) were collected regularly or as required in 47 L gas cylinders. The gaseous composition was analysed with gas chromatography equipped with a thermal conductivity detector (GC-TCD, Agilent 490). The major components were CO_2 (95 ± 1.5 %), N_2 (4 ± 1 %), and O_2 (1 ± 0.2 %). The presence of a minute quantity of O_2 could be due to errors in the sampling or sample injection processes. Moreover, there were also hydrocarbon traces (around 130 ppb) in the brewery gas (Roy et al., 2021a).

3.2.2.3 MES Reactor setup and acclimatization experiments

All MES experiments were carried out in triplicate at 28 ± 2 °C in custom-made two-chamber cubic reactors made up of polytetrafluoroethylene (PTFE) with a total empty bed volume of 1.12 L (working volume of each chamber 0.5 L). A dimensionally

stable graphite plate (40 cm^2) and mixed metal oxide coated titanium plate were used as cathode and anode, respectively. A cation exchange membrane (117 Nafion; Sigma Aldrich) separated the anode and cathode chambers. The same minimal medium prepared anaerobically mentioned in 3.2.2.1 but lacking 2-bromoethanesulfonate and L-cysteine served as catholyte, and 0.5 M Na_2SO_4 at pH 2.5 (adjusted with 1 M H_2SO_4) was used as the anolyte. Abiotic electrochemical water oxidation and microbial CO_2 reduction were the desired reactions in the anode and cathode chambers, respectively. The MES experiments were conducted in a three-electrode configuration mode under potentiostatically-controlled conditions using Potentiostat/Galvanostat (VSP300, BioLogic Science Instruments, France). All the electrode potential data are reported against Ag/AgCl (3.5 M KCl) reference electrode (0.205 V vs. SHE). Biotic open circuit potential (OCP) control (without an energy source) and the abiotic electrochemically connected control (without microbial inoculum) experiments were conducted to confirm the electricity-driven microbial CO_2 reduction process in the MES reactors.

Before conducting the main MES experiments, the cathode was polarised overnight at − 0.6 V for electrochemical activation of the electrodes, membrane, and electrolyte. After that, to develop a stable performing biocathode, acclimatization was performed for two batch cycles with unpurified brewery CO_2 as the sole carbon source (fed for 30 mins every day at 1 L/d feed rate) at a constant cathode potential of − 1.0 V vs. Ag/AgCl in a fed-batch mode. The cathode chambers were inoculated with an active enriched mixed culture with 0.25 OD_{600}. The MES experiments were conducted in two experimental phases, as elaborated in subsequent sections, using the set up shown in Figure 3.2.1.

3.2.2.4 MES Experimental phases

3.2.2.4.1 Brewery CO_2 feed rate and $E_{cathode}$ optimization

Following the completion of two batch cycles of acclimatization, unpurified brewery CO_2 was supplied in a continuous mode with the help of mass flow controllers (Alicat/MCM-100SCCM-D) at various feed rates, i.e., 0.9, 0.7, 0.4 and 0.3 L/d in the cathode chamber to check the effect of different gas feed rates. The mass flow controllers were calibrated by the manufacturer using air. After the gas feed rate optimization, the $E_{cathode}$ was varied at three levels, viz., − 1.2, − 1.0, and − 0.8 V vs Ag/AgCl.

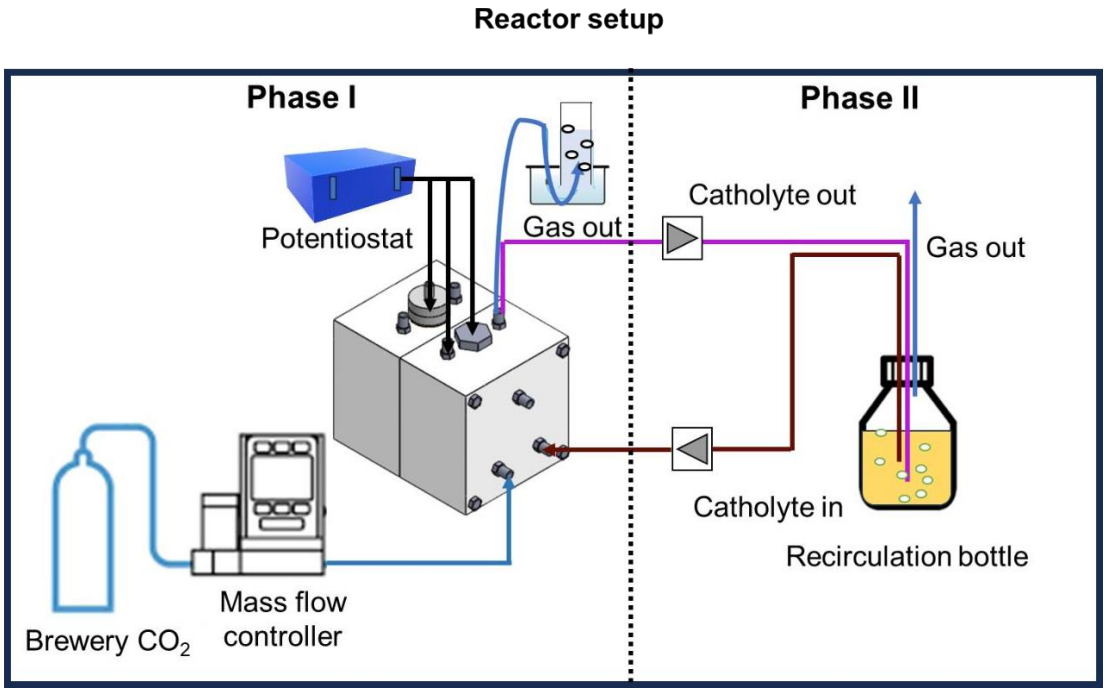

Figure 3.2.1: Schematic diagram of the MES experimental setup and phases. In phase II, the catholyte recirculation loop was introduced to the setup.

3.2.2.4.2 Catholyte recirculation impact on MES productivity

The catholyte recirculation loop was introduced in the subsequent stage following the optimization of the brewery gas feed rate and $E_{cathode}$ parameters (Figure 3.2.1). Recirculating the catholyte can offer advantages such as improved mixing, thereby enhancing the contact between microbial cells and substrates, increased nutrient availability, and better retention of gases, which ultimately helps minimize mass transfer limitations.During this condition, the gas was fed continuously at a rate of 0.7 L/d and $E_{cathode}$ was set to − 1 V. A recirculation bottle (0.5 L) hosting an additional 240 mL medium was assembled with the cathode chamber of MES reactors. The resulting total catholyte volume was 780 ± 20 mL in these experiments. The catholyte was recirculated in batch mode at a feed rate of 10 mL/min with the help of a peristaltic pump (Cole-Parmer). This recirculation rate was selected as it was the lowest at which continuous recirculation could be carried out successfully without any operational issues in this system. Every other operating condition remained unchanged.

3.2.2.5 Analysis and calculations

Gas and liquid samples were analysed at regular intervals of two days. As microbial growth and organic production are directly linked with the microbial CO_2 fixing capabilities, liquid samples were analysed to evaluate growth in the bulk phase by monitoring turbidity (OD_{600}) using UV–VIS spectrophotometer (Photo-lab 7600) and the bioproduction of the organics (C1-C4 acids and alcohols by HPLC (Agilent 1260 Infinity II, RID Detector, Hiplex H column). The biomass in the bulk phase was estimated by analysing insoluble COD (Rice et al., 2012). The gas samples were analysed for H_2, CO_2, O_2 and H_2S with GC-TCD (Agilent 490 Micro GC) as described elsewhere (Roy et al., 2021a). The key production indicators like production rates, coulombic (electron recovery into products), and energetic (energy recovery into products) efficiencies were calculated according to protocols described elsewhere (Patil et al., 2015b). The carbon recovery efficiency (i.e., CO_2 to acetic acid) was calculated, as mentioned elsewhere (Das and Ghangrekar, 2018).

Apart from these, cyclic voltammetry (CV) analysiswas conducted to achieve a deeper insight into the redox active components of the system. At a constant scan rate of 1 mVs^{-1}, cyclic voltammetric analysis was carried out for two cycles within the potential window of − 1.4 V to − 0.2 V at three distinct experimental conditions: before microbial inoculation, after inoculation, and at the end of the experiment to better understand the role of microbes in enhancing the hydrogen evolution electrocatalytic process.

3.2.3 Results and Discussion

3.2.3.1 Development of stable biocathodes through a batch mode operation

First, unpurified brewery CO_2 was fed into the cathode chamber as the sole carbon source in a batch mode to develop a stable-performing CO_2-fixing microbial consortium in the MES reactors. In order to identify a clear trend and better insights into the process, the experiments were conducted for more than two weeks under each scenario. Following two batch cycles, constant acetic acid production was detected, indicating the formation of a stable biocathode (Figure 3.2.2) (Marshall et al., 2012, Patil et al., 2015a). Immediate growth and acetic acid production were observed as

previously enriched mixed cultures were used as the inoculum. Acetic acid was the major organic detected in the MES reactors. The acetic acid titer and growth increased after three days of microbial inoculation in the cathode chamber of the MES reactors (Figure 3.2.2). After eighteen days, it entered a stationary phase with 1.6 ± 0.37 g/L of acetic acid. This production was achieved at the end of the second batch cycle. These observations confirmed the growth and establishment of a stable, active CO_2-fixing microbial community at the cathode and in the catholyte.

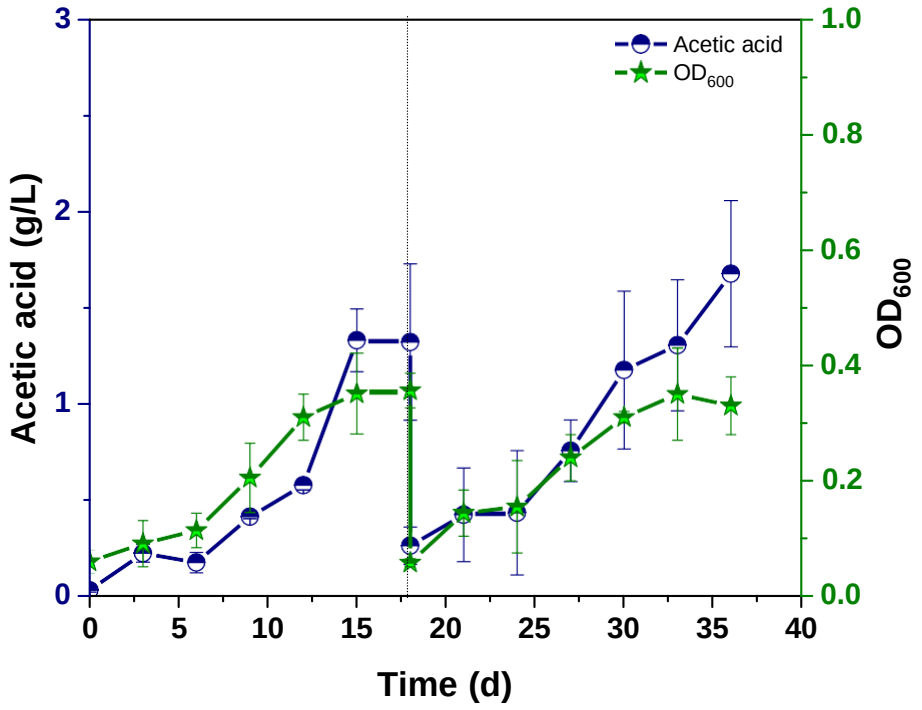

Figure 3.2.2: Acetic acid production and OD_{600} profiles of the microbial electrosynthesis process with brewery CO_2 feed during the acclimatization period.

3.2.3.2 Brewery CO_2 feed rate optimization at a continuous gas feeding condition

At the lower feed rate of 0.3 L/d, a maximum acetic acid titer of 1.94 ± 0.44 g/L was attained with a production rate of 0.102 ± 0.02 g/L/d. A clear link between microbial growth and acetic acid titer was observed, which is seen in the microbes that follow the Wood–Ljungdahl pathway for CO_2 fixation (Ragsdale, 2008). After the 19th day, when acetic acid production stabilized or levelled off as the cycle reached the stationary phase, 90 % of the catholyte was replaced with a fresh medium, and the gas feeding was increased to 0.4 L/d. As a result, the acetic acid titer reached up to 4.9

g/L. With a further increase in gas feed rate to 0.7 L/d, the acetic acid production increased to 6.6 ± 0. 4 g/L at a production rate of 0.44 ± 0.03 g/L/d (Figure 3.2.3). The coulombic efficiency of 85 ± 23 % in acetic acid was achieved after the 15th day of the cycle at 0.7 L/d brewery CO_2 feed rate. Compared to the lower feed rate of 0.3 L/d, a more than threefold increase in the acetic acid titer was achieved at 0.7 L/d feed rate. With a further increase in gas feed rate to 0.9 L/d, the acetic acid titer did not increase further as it stabilized at 5.5 ± 0.21 g/L. This decrease in acetic acid titer can be attributed to the rapid removal of H_2 produced in the cathode chamber at the high gas feed rate, thereby hampering the H_2-mediated electron transfer and bioproduction process.

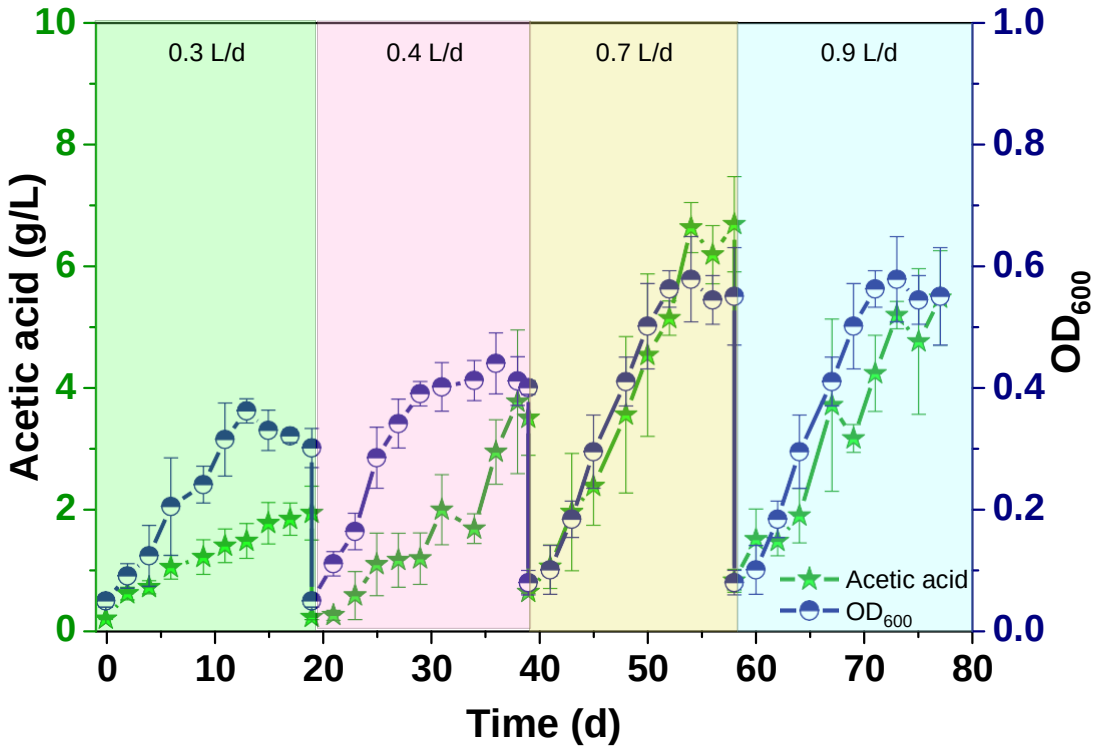

Figure 3.2.3: Microbial growth (as OD_{600}) and acetic acid production profiles in the MES reactors fed with unpurified brewery CO_2 continuously at varying feed rates.

Neither microbial growth nor organic acid production was observed in the control reactors (Figure 3.2.4). This indicates that microbes were primarily responsible for catalysing the CO_2 reduction, and no other energy source, apart from electricity, was provided in the MES reactors.

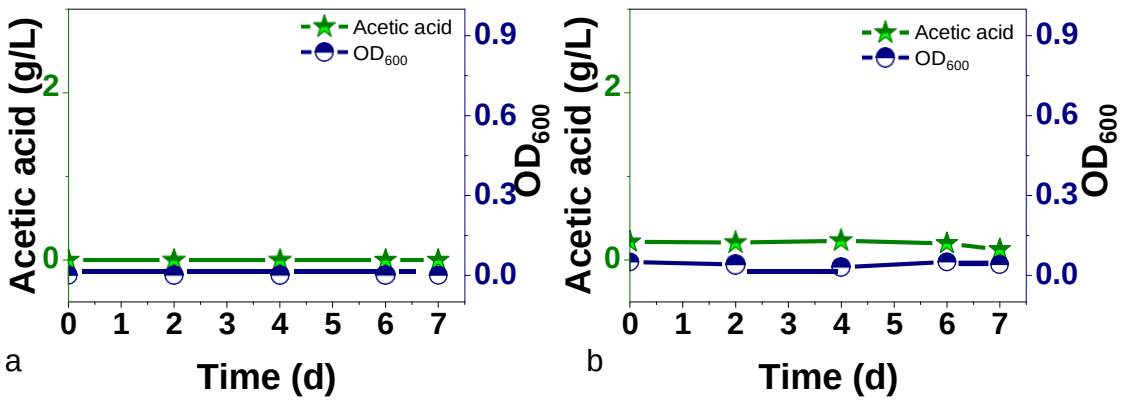

Figure 3.2.4: Acetic acid production and OD_{600} profiles of the control experiments: a) Abiotic electrochemically-connected and b) Biotic electrochemically-unconnected or open circuit reactors.

3.2.3.3 $E_{cathode}$ optimization at a continuous brewery CO_2 feed condition

After optimizing the gas feed rate (section 3.2), the effect of the cathode potential was analysed at the constant brewery CO_2 feed rate of 0.7 L/d. Residual acetic acid was present from the previous cycles. Depending on the H_2 evolution potential (section 3.4), the highest cathode potential was set at – 0.8V, and it was further varied to – 1 and – 1.2 V. After reaching acetic acid titer of 6.6 g/L at – 1 V, the higher cathode potential of – 0.8 V was applied. A considerable decrease in the growth and acetic acid production was prominent, reaching only about 1.1 g/L after 15 days (Figure 3.2.5). Even after 21 days, the acetic acid production and OD_{600} remained at 1.15 ± 0.52 g/L and 0.38, respectively. This low performance can be attributed to the limited H_2 production in the cathode chamber. Therefore, the cathode potential was switched to a lower value of – 1.2 V in the next stage. As a result, production as well as microbial growth started to improve readily. The acetic acid titer reached 3.8 ± 0.75 g/L after 14th day of incubation. The maximum acetic acid titer reached 4.7 ± 0.86 g/L with a production rate of 0.23 ± 0.03 g/L/d and CE of 57 ± 23 % in this condition. The primary reason for this was the prevalent excess production of H_2, which the microbes were not able to utilize efficiently. Consequently, a significant portion of electrons was recovered in H_2 rather than acetic acid. Taken together, the findings suggest the cathode potential of – 1 V vs. Ag/AgCl was the most efficient for brewery CO_2 utilization at 0.7 L/d feed rate. This observation is in agreement with the previous studies wherein at an $E_{cathode}$ of – 1 V, both the production of organics, specifically

acetic acid, as well as biofilm formation with gaseous CO_2 (as the sole carbon source) were better compared to lower or higher $E_{cathode}$ (Izadi et al., 2020, Das et al., 2020).

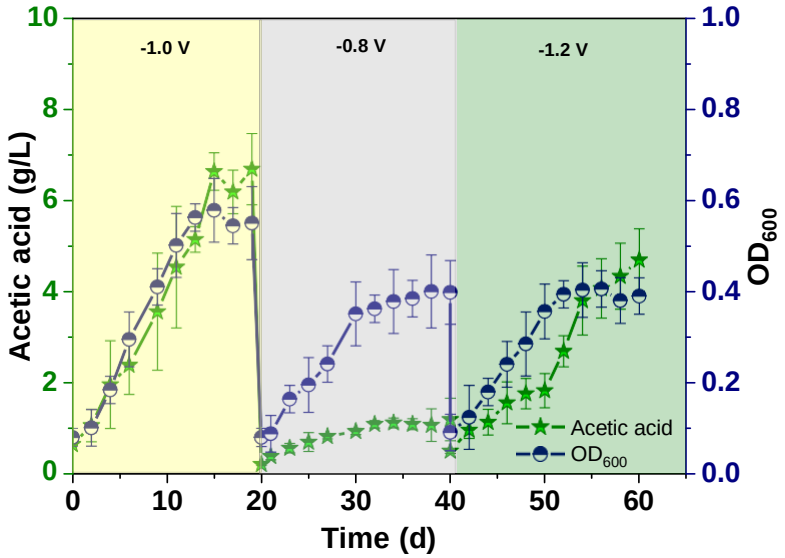

Figure 3.2.5: Microbial growth (as OD_{600}) and acetic acid production profiles in the MES reactors fed with unpurified brewery CO_2 (at 0.7 L/d gas feed rate) at varying $E_{cathode}$.

3.2.3.4 Electrochemical assessment of the biocathode

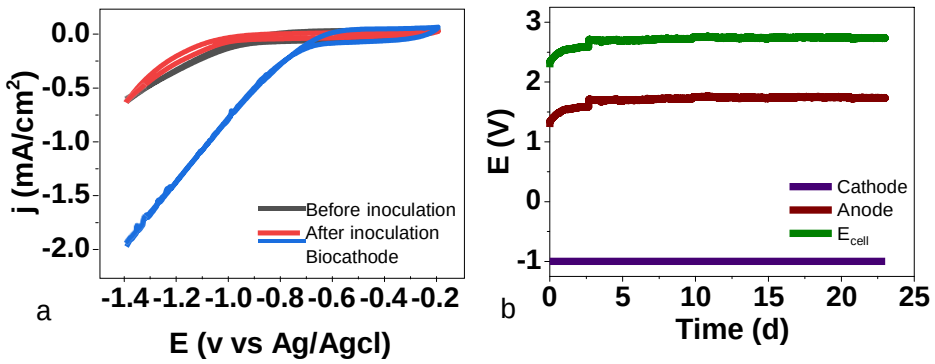

Figure 3.2.6: Representative cyclic voltammograms recorded at the cathode (a) and potential profiles (b) of the MES reactors operated at the gas feed rate of 0.7 L/d and $E_{cathode}$ of −1 V.

Similar trends in electrochemical parameters were observed in all the operational conditions described in sections 3.2.3.2 and 3.2.3.3. Therefore, the representative electrochemical data of one of the reactors that showed the best acetic acid productivity at the optimized condition of gas feed rates of 0.7 L/d and $E_{cathode}$ of – 1 V is shown in Figure 3.2.6. The before and after microbial inoculation CVs indicated

the absence of redox-active moieties at the cathode surface and in the bulk phase (or catholyte medium). The peaks and H_2 evolution potential were identified using the first-order derivative of a cyclic voltammogram. A substantial shift from − 0.804 V (before/after inoculation condition) to − 0.55V in the H_2 evolution potential was evident in the biocathode CV. It implies that the H_2 evolution overpotential at the biocathode decreased substantially (about 0.25 V) than at the abiotic cathode (Figure 3.2.6a). Moreover, the cathodic reduction current increased remarkably in the case of the biocathode (e.g., − 0.8 mA/cm^2 at biocathode vs. − 0.09 mA/cm^2 at abiotic cathode at − 1 V). Therefore, it is evident that the microbial catalysts at the cathode chamber of MES systems enhanced the electrocatalytic activity by lowering the H_2 evolution overpotential and increasing the cathodic current draw. These observations are consistent with the previous MES studies conducted with pure or industrial CO_2 (Jiang et al., 2013, Jourdin et al., 2015a, Patil et al., 2015a, Bajracharya et al., 2015, Roy et al., 2021a, Roy et al., 2021b).

The representative potential profiles of MES reactors operated at gas feed rates of 0.7 L/d and $E_{cathode}$ of – 1 V are shown in Figure 3.2.6b. An E_{cell} of 2.8 ± 0.3 V and 2 ± 0.16 V was observed in the case of − 1.2 and − 0.8 V applied cathode potential, respectively. The best MES performance was observed at 2.9 ± 0.64 V with an $E_{cathode}$ of − 1 V and gas feed rate of 0.7 L/d. The E_{cell} is comparable with that of a smaller-scale H-shaped reactor routinely used in MES studies (Roy et al., 2021a, Rojas et al. 2018b). The electrochemical data unambiguously reveals that the anode potential, which was often around 1.77 V compared to the cathode potential, which was fixed at – 1 V, was majorly responsible for increasing the overall cell voltage. Other advantageous and low-energy demanding anodic processes can be employed to handle the high overpotential at the anode, lowering the total energy consumption of the process (Verma et al., 2019, Battle Vilanova et al., 2019).

3.2.3.5 Catholyte recirculation enhanced acetic acid production

Introducing a recirculation bottle for catholyte in the MES setup had a notable effect on the production, growth, and pH profiles, as outlined in Figure 3.2.7. Substantial growth and production were observed early in the operation, indicating the effectiveness of this setup. Around the seventh day of operation, the cycle entered the log phase, marked by a pronounced spike in acetic acid production. During the initial

nine days, when the acetic acid titer was around 3 g/L, no significant pH drop was observed. However, after ten days, a noticeable pH drop was observed due to the accumulation of product (acetic acid). Eventually, the maximum acetic acid titer reached 7.6 ± 0.65 g/L after 15 days, with a production rate of 0.5 ± 0.03 g/L/d. Therefore, it is evident that catholyte recirculation was beneficial for the overall enhancement of the process.

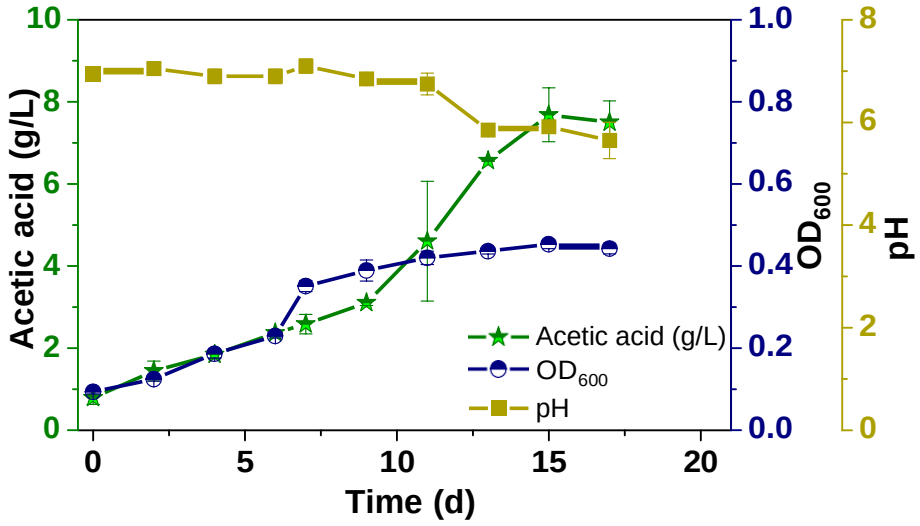

Figure 3.2.7: Microbial growth (as OD_{600}), pH, and acetic acid concentration profiles of the MES reactors equipped with the catholyte recirculation loop and operated at the gas feed rate of 0.7 L/d and $E_{cathode}$ of – 1 V.

3.2.3.6 MES performance assessment at the optimized set of conditions

Different secondary process parameters like carbon recovery efficiency (CRE), CE, energetic efficiency (EE), and production rates are crucial in assessing the performance of MES with unpurified brewery CO_2 (Table 3.2.1). Though a higher gas feed rate is beneficial for more carbon availability in the system, it can lead to removing H_2 from the cathode chamber, thereby impeding the H_2-dependent acetic acid production. On the other hand, $E_{cathode}$ optimization is necessary in order to maximize the production of the desired organic compounds via MES (Das et al., 2020). At higher $E_{cathode,}$ H_2 production becomes one of the limiting factors resulting in compromised acetic acid production and carbon recovery efficiency (3 %). However, it proves beneficial in terms of coulombic (97 %) and energetic efficiencies (52 %). Conversely, lower $E_{cathode}$ values enhance production, but low energy and

electrons are less recovered in acetic acid. With the optimized brewery feed rate of 0.7 L/d and applied cathode potential of − 1 V, a maximum acetic acid titer of 6.6 ± 0.4 g/L was achieved at a production rate of 0.44 ± 0.02 g/L/d, with a CE of 85 %, CRE of 24.5 % and EE of 34 %. Therefore, establishing an optimum balance between the flow rate and $E_{cathode}$ is crucial in order to achieve enhanced production in continuously fed systems.

With the catholyte recirculation loop, there was around a 16 % increase in acetic acid titer. A similar improvement (in product titer) has been reported for gas recirculation as well in a previous study by Mateos et al. (2019). Additionally, recirculation led to improvements in CE (92 %) and EE (34 %) of the overall process, along with a 31 % increment in CRE. These productivity enhancements can be attributed to the improved mixing and prolonged retention of CO_2 and H_2 in the MES system. Similar positive trends were observed in other studies as well, where only the headspace gas was recirculated in the cathode chamber (Mateos et al., 2019, Bajracharya et al., 2022).

Table 3.2.1 Comparison of key production parameters of MES with brewery CO_2 at different operational conditions.

Parameters	**Brewery CO_2 feed rate (L/d)**				***Applied cathode potential / $E_{cathode}$ (V)***			***Catholyte recirculation condition***
	0.3	**0.4**	**0.7**	**0.9**	**− 0.8**	**− 1**	**− 1.2**	**− 1V and 0.7 L/d**
Product titer (g/L)	1.94 ± 0.44	3.7 ± 1.1	6.6 ± 0.4	5.5 ± 0.21	1.15 ± 0.5	6.6 ± 0.4	4.7 ± 0.7	7.68 ± 0.65
Production rate (g/L)/d)	0.102 ± 0.02	0.12 ± 0.06	0.44 ± 0.03	0.3 ± 0.04	0.05 ± 0.02	0.44 ± 0.03	0.23 ± 0.03	0.5 ± 0.04
Coulombic efficiency (%)	61 ± 13	82 ± 18	85 ± 23	78 ± 13	97 ± 19	85 ± 23	57 ± 23	92 ± 4
Energetic efficiency (%)	27 ± 6	33.8 ± 5	34.5 ± 14	31 ± 5	52 ± 10	34.5 ± 14	21 ± 7	34 ± 2
Carbon recovery efficiency (%)	15.5 ± 3.5	20 ± 6	24.5 ± 1.5	14 ± 1.6	3 ± 1	24.5 ± 1.5	13 ± 2	44 ± 4
E_{cell}	2.7 ± 0.5	2.7 ± 0.15	2.98 ± 0.64	2. 28 ± 0.25	2 ± 0.2	2.98 ± 0.64	2.82 ± 0.27	2.9 ± 0.44

Understanding the magnitude of productivity improvement derived from liquid recirculation is pivotal for the rational design and operation of scaled-up systems despite the accompanying expenses linked to pump usage. While recirculation entails additional costs, it proves advantageous for the overall productivity of the MES system. Hence, its integration with the MES reactors is justified (Bian et al., 2024, Arends et al., 2017, Rovira-Alsina et al., 2022, Cui et al., 2023, Deutzmannet and Spormann, 2024).

3.2.4. Conclusions

The findings of this study validate the feasibility of MES technology for producing acetic acid using unpurified brewery CO_2, a plentiful but underexplored carbon source. Maintaining an optimal balance between gas flow rate and cathode potential is imperative for efficient H_2-mediated production. Recirculating the catholyte medium enhances acetic acid production by improving mixing and prolonging the retention of gaseous substrates. Nonetheless, the energy-intensive anodic reaction remains one of the major constraints of the MES process. Further research should prioritize addressing this challenge.

Chapter 4

Testing and scalability assessment of microbial electrosynthesis for biogas upgradation

This chapter pertains to the assessment of the applicability and scalability of the microbial electrosynthesis process for biogas upgradation through the following two major activities.

4.1 Assessment of microbial electrosynthesis for biogas upgradation through CO_2 conversion into acetic acid

4.2 Assessment of the scale-up and techno-economic feasibility of the microbial electrosynthesis process for biogas upgradation

4.1: Assessment of microbial electrosynthesis for biogas upgradation through CO_2 conversion into acetic acid*

*The contents of this sub-chapter are adapted with permission from the following peer-reviewed journal publication:

Roy M., Yadav S., Patil S. A. Biogas Upgradation Through CO_2 Conversion into Acetic Acid via Microbial Electrosynthesis. Frontiers in Energy Research, 2021, 9: 723. doi.org/10.3389/fenrg.2021.759678.

4.1.1 Introduction

According to the World Energy Forum, fossil fuel sources will be exhausted by the next 10 decades due to the unquenchable global energy demand (Weiland, 2010, Sahota et al., 2018). Since energy is the pillar of globalization, its demand is increasing exponentially with time. Under these circumstances and for sustainable growth, immediate measures need to be taken to rapidly implement renewable energy. Among various sources, biogas is a promising source for meeting the world‘s importunate energy demand. It has great potential to evolve as an alternative fuel for vehicles or to meet the ever-growing electricity demand and is increasingly gaining preference throughout the globe. For example, CNG-fueled buses are already operating in the USA. Over 2200 digesters are currently operational for biogas production in the USA (Biogas Industry Market Snapshot | American Biogas Council). Due to the price increase of gasoline in Europe and North America, more inclination is visible for biomethane (Engerer and Horn, 2010). About 42 million biogas plants produce around 13 million m^3 biogas in China. By the end of 2030, Germany will be producing nearly 10 billion m^3 biogas. In India,4.9 million digesters with a 2 million m^3 biogas production capacity are present (Thiruselvi et al., 2021). India has planned to target renewable energy at 275 gigawatts by the end of 2027 (Thiruselvi et al., 2021). The Indian Government has also taken several initiatives like the Sustainable Alternative toward Affordable Transportation (SATAT) initiative to secure the off-take of compressed biomethane, the National Policy on Biofuels (2018) for financing as well as fiscal incentives, and the Motor vehicles rule to promote the usage of BioCNG in motor vehicles. Climate Change Levy of UK and Finland provide tax exemption for energy from renewable sources. These data suggest the prominence of biogas in renewable energy development programs across the globe.

The key challenge in using biogas as fuel is its low calorific value due to the presence of CO_2. For instance, the upgraded BioCNG (around 52,000 kJ/kg) has around 2.5-fold higher calorific value than untreated biogas containing ~ 55 % methane (around 19500 kJ/kg) (Dere et al., 2017). Hence, biogas upgradation is essential to make it a high-quality fuel. To this end, several technologies based on absorption, adsorption, and membrane separation processes have come up over the years (Thiruselvi et al., 2021, Sahota et al., 2018, Kadam and Panwar, 2017, Angelidaki et al., 2018). Water scrubbing is the most feasible, but it is not considered economical and sustainable due

to high capital costs and freshwater requirements. Apart from water scrubbing, pressure swing absorption and chemical adsorption are also established techniques, but they are complex processes with high investment costs (Sahota et al., 2018). More sustainable ways of biogas upgradation are thus constantly explored. Microbial electrosynthesis (MES) is one of the microbial electrochemical technologies (METs) in which electricity-driven CO_2 reduction is enabled with the help of microbial catalysts. Based on the nature and extent of electrochemical interactions between the working electrode and microbial catalysts, METs are broadly categorized into two groups, namely primary and secondary METs (Schröder et al., 2015). In primary METs, a prominent functional connection between the microbial catalysts and the working electrode exists via direct or mediated electron transfer. In secondary METs, electrochemistry is indirectly linked to the microbial process, for instance, through the electrochemical control of parameters such as metabolite concentration and pH. In most MES processes, including the present study, microorganisms catalyze the target reactions via direct and/or mediated electron transfer mechanisms by forming biofilm at the cathode surface and planktonic growth in the bulk phase (Patil et al., 2015a, Labelle et al., 2020). Using this technique, CO_2 from the biogas can be converted to value-added products like methane, acetic acid, butyric acid, propionic acid, and the methane concentration can be enhanced. The most attractive part of the MES process is the CO_2 utilization instead of just removal. In one approach, the microbial electrolysis process is integrated with biogas plants, and CO_2 is microbially reduced to methane. Significant work has been done on coupling MES with anaerobic digestion (AD) with different reactor designs and operational conditions (Wang et al., 2021, Sravan et al., 2020). Biogas is fed to the MES reactors in another approach, and CO_2 is reduced to methane through hydrogenotrophic, acetoclastic, or methylotrophic pathways (Evans et al., 2019).

Limited work has been done on utilizing CO_2 from biogas to produce organic acids or other products via MES (Das and Ghangrekar, 2018, Das et al., 2018, Jourdin et al., 2015b, Kokkoli et al., 2018). The previous MES studies with biogas feedstock mostly focused on organics formation, specifically acetic acid, and barely on enhancing methane content. The present study aimed to investigate the applicability of MES for biogas upgradation through CO_2 conversion into acetic acid. After establishing a batch mode process, the effect of different biogas feed rates was studied for the

upgradation of biogas along with the production of acetic acid. The cyclic voltammetry and the metagenomic analyses of the biocathode were conducted to shed light on the bioelectrochemical processes and microorganisms involved in the bioproduction process.

4.1.2 Methodology

4.1.2.1 Microbial inoculum source and cultivation conditions

A previously enriched chemolithoautotrophic mixed microbial culture dominated by *Acetobacterium* spp. was used as the inoculum source (Roy et al., 2021a). For the MES experiments, a minimal medium with pH 7 was used. It contained K_2HPO_4 (5.35 g/L), KH_2PO_4 (2.62 g/L), NH_4Cl (0.25 g/L), KCl (0.5 g/L), $CaCl_2{\cdot}2H_2O$ (0.15 g/L), $MgCl_2{\cdot}2H_2O$ (0.6 g/L), trace metal solution (1 mL/L), selenium-tungstate solution (1 mL/L), vitamin solution (2.5 mL/L), 2-bromoethanesulfonate (6.4 g/L), $Na_2S{\cdot}9H_2O$ (0.3 g/L), and resazurin (0.5 mL/L from a 0.1% stock solution) (Roy et al., 2021a). It was cultivated and maintained with CO_2 and H_2 (H_2:CO_2 ratio was 4:1) as the sole carbon and energy sources, respectively, under anaerobic conditions at 28 ± 2 °C. An active culture was inoculated in the cathode chamber of MES reactors to have 0.25 OD_{600} in suspension during the start-up phase.

4.1.2.2 Biogas sampling and characterization

The biogas was collected from an operational and well-maintained anaerobic sewage treatment plant (Phase 3, Panchkula, Haryana). A vacuum pump (Model N86 KT.45.18; KNF pump) was used to collect biogas in 6 L passivated SilcoCan steel canisters (Restek, USA). A Teflon gas tubing was used to collect the biogas as it can withstand high temperatures (> 180 °C) and pressure (> 40 psi). A PTFE filter (0.2 µm) was used in the front of the tubing to restrict the particulate matter from the biogas from entering the canister. At the same time, the moisture content was minimized with the help of a moisture trap installed in gas tubing where magnesium perchlorate was used as the moisture-absorbing reagent. The biogas samples were analyzed by GC with TCD (Agilent 490 Micro GC, section 4.1.2.5). The major components of biogas were methane (60 ± 2 %), CO_2 (28 ± 5 %), and N_2 (10 ± 2 %). A trace amount of H_2S was also present in the sample. 1 ± 0.1 % O_2 was also observed, which was most likely present due to manual sampling error.

4.1.2.3 MES reactor setup and experiments

Custom-made double-chambered glass reactors with an empty bed volume of 700 mL (350 mL in each chamber) were used for this study. The catholyte was the same as mentioned in section 4.1.2.1, and the anolyte was 0.5 M Na_2SO_4 with pH 2.5 (adjusted with 1 M H_2SO_4). The experiments were conducted in three-electrode configuration mode under potentiostatically controlled conditions (VSP300, BioLogic Science Instruments, France). The working volume was 250 mL. A graphite plate with 10 cm^2 and a dimensionally stable mixed metal oxide (MMO) coated titanium plate with 7.5 cm^2 projected surface area were used as working (cathode) and counter (anode) electrodes, respectively. The anode and cathode chambers were separated by a 117 Nafion proton exchange membrane (Sigma-Aldrich). Water oxidation and CO_2 reduction take place in the anode and cathode chamber, respectively. Continuous biogas sparging helped to limit the oxygen (produced in the anode chamber) migration to the cathode chamber (below 1%). The cathode material and proton exchange membrane were pretreated as described elsewhere (Roy et al., 2021a). All MES experiments were performed in duplicate at an incubation temperature of 28 ± 2 °C. The performance of the duplicate reactors for reported parameters was reproducible and very close; therefore, average data has been presented in all the figures. The electrode potential data are reported against Ag/AgCl (3.5 M KCl) reference electrode (0.205 V *vs.* SHE). The experimental setup and the reactions in MES reactors are illustrated in Figure 4.1.1.

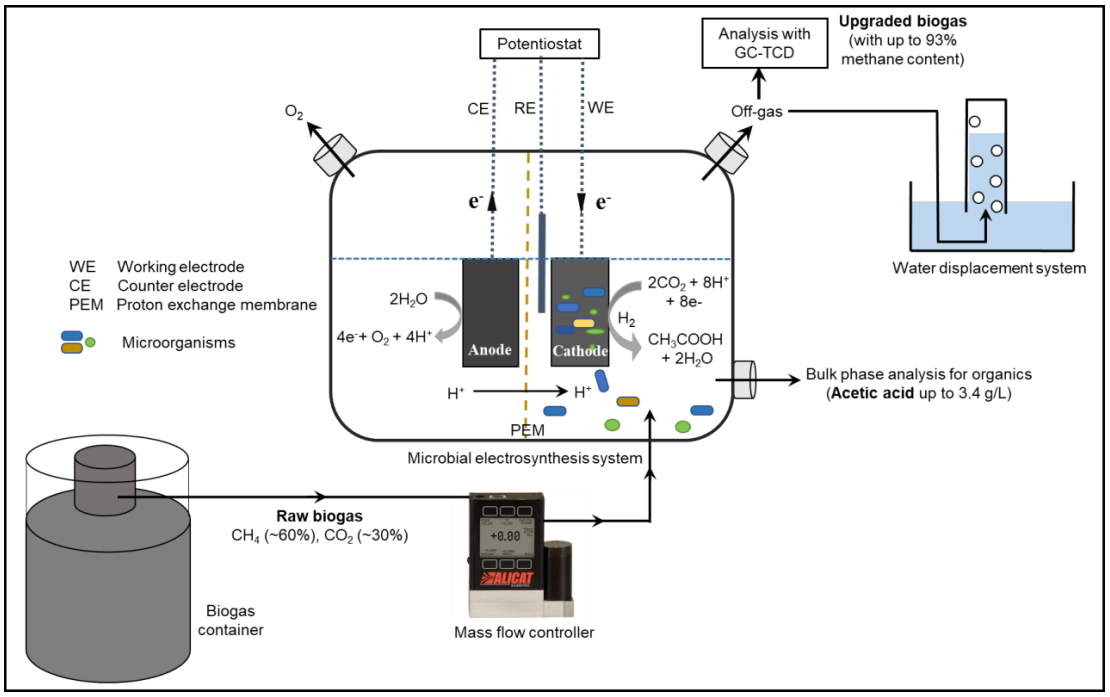

Figure 4.1.1: Schematic diagram of the experimental setup used for biogas upgradation in this study.

Two types of control experiments were conducted. These include the biotic open circuit potential (OCP) experiment (with all media components and carbon source but not electrically connected, i.e., no electron/energy source) and the abiotic connected (with all the components and electrically connected but uninoculated) experiment.

Activation polarization was performed at − 0.6 V before inoculation or starting the main experiment for electrochemical activation of the electrodes, membrane, and electrolyte. A constant potential of − 1.0 V *vs.* Ag/AgCl was applied at the cathode to facilitate H_2-based bioproduction by using the chronoamperometry (CA) technique. The reduction current response was also monitored at a constant time interval (5 min). Cyclic voltammetric analysis was conducted for two cycles (data of the 2nd cycle has been reported) within a potential window of − 1.4 and − 0.2 V at a constant scan rate of 1 mVs^{-1} at different experimental conditions, i.e., before inoculation, after inoculation, and at the end of each biogas feed condition. The data of the second cycle was used for analysis.

4.1.2.4 Biogas feeding

In the first phase, 30 mL of CO_2-containing biogas (with the composition stated in section 4.1.2.2) was added to the catholyte daily to establish a stable performing

biocathode. The CO_2 present in the biogas gets dissolved in the form of bicarbonate at pH 7. This was the sole carbon source for the microbes. A gas displacement assembly was attached to the reactor's outlet to maintain the pressure condition inside the reactors. After the completion of two batch cycles, the biogas was fed in a continuous flow mode at different feed rates, *viz*., 0.5, 0.3 and 0.2 mL/min, using the mass flow controllers (Alicat / MCM-100SCCM-D) in the second phase. The MFC calibration was carried out with the air by the manufacturer (Alicat Scientific). The correction factors were applied for different gases depending on their viscosity, density, and compressibility. The standards used for calibrations were NIST (National Institute of Standards and Technology) traceability. The volumetric flow of the off-gas from MFCs was measured to confirm the flow rates with the help of a water displacement assembly, and it was found to be the same. The volumetric flows of the off-gas from the microbial electrosynthesis reactors were also measured intermittently at each experimental condition and were found to be marginally lower than the applied feed rates. For instance, in the case of 0.5 mL/min biogas feed rate, the off-gas flow was about 0.47 mL/min. Similar trends were observed in other gas feed rates. The effect of different feed rates was evaluated on methane content increase as well as acetic acid production after acclimatization of the reactors for at least two days at each feed rate.

4.1.2.5 Analysis and calculations

The bulk phase and gas samples from MES reactors were taken at regular intervals of two days. To assess the gas composition in the headspace and to know the methane concentration, the gas analysis was done by GC-TCD (Agilent 490 Micro GC) 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]. The bulk phase organics (C1-C4 organic acids and alcohols) analysis by HPLC (Agilent 1260 Infinity II, RID Detector, Hiplex H column, 5 µM H_2SO_4 as mobile phase, flow rate 0.5 mL/min, Temperature 50 °C) was performed to evaluate the efficiency of microbes to produce organics from CO_2 in biogas. Additionally, monitoring of microbial growth via OD measurements at 600 nm (Photo-lab 7600 UV-VIS spectrophotometer) and pH (Oakton PC2700) in the bulk phase was done. Different parameters, such as product titer, production rates (normalized by cathode surface area and catholyte volume), coulombic efficiency (electron recovery into

organic products), carbon recovery efficiency (Das and Ghangrekar, 2018) and energetic efficiency were calculated as described elsewhere (Patil et al., 2015b). Since the increase in methane content is not directly related to energy consumption in this study, we did not consider it in CE and EE calculations.

4.1.2.6 Microbial community analysis

To understand the microbial communities developed at the biocathode and in the bulk phase, V3-V4 16S amplicon sequencing-based analysis was conducted. For this, the genomic DNA of the inoculum source and from two duplicate MES reactors named R1 and R2 (biocathode and bulk phase) was isolated on the completion of the electrosynthesis experiments using QIAGEN DNeasy PowerSoil Pro Kit. Further DNA quantification was done by Nanodrop (Genova Nano – 4359). Agarose gel electrophoresis (1 %) was also performed to assess the integrity of the isolated genomic DNA. Since V3-V4 regions of 16S rRNA are the most conserved regions, they were amplified using specific V3 Forward (341F: 5′-GCCTACGGGNGGCWGCAG-3′) and V4 Reverse (805R: 5′-ACTACHVGGGT ATCTAATCC-3′) primers. Again 2 % agarose gel electrophoresis was performed to analyze the amplified products. Later, Illumina MiSeq 2500 platform at Eurofins Genomics India Pvt. Ltd (Bangalore, India) was used for amplicon sequencing. Trimmomatric v0.38 was used for trimming the adaptor and primer sequences. FLASH v1.2.11 was run for alignment as well as for selecting the operational taxonomic unit (OTU). At last, the OTU products were run through the Silva database to find the most similar OTU hits using the Qiime1 pipeline and R packages for visualization. The raw amplicon sequences have been submitted to the NCBI short read archive under bio-project PRJNA659908.

4.1.3 Results and Discussion

4.1.3.1 Biocathode development through batch mode operation

In order to form an efficient biocathode for CO_2 utilization, the biogas was first supplied in a fed-batch mode. Since the enriched mixed culture contained chemolithoautotrophic microbes (Roy et al., 2021a), immediate organics production, in particular, acetic acid, was detected from the start of the experiment (Figure 4.1.2).

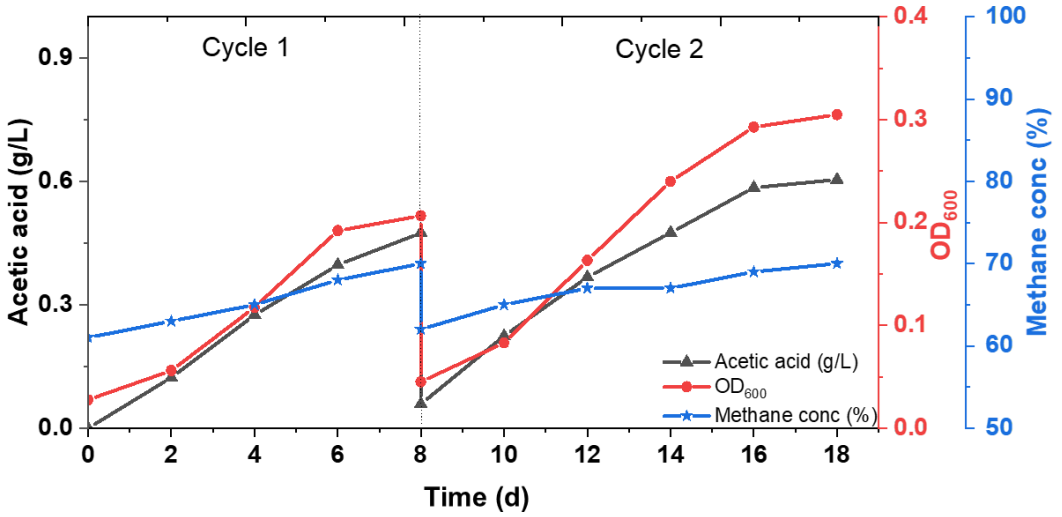

Figure 4.1.2: Microbial growth (in terms of OD_{600}), acetic acid and methane concentration profiles of the MES reactors fed with biogas in batch mode experiments.

Since the inoculum source contains mainly *Acetobacterium* spp. that follow the Wood-Ljungdahl pathway for CO_2 fixation, microbial growth and the acetic acid concentration are directly correlated. The maximum titer achieved was 0.6 ± 0.1 g/L with a production rate of 0.06 ± 0.001 g/L/d after eight days (Figure 4.1.2). Along with the acetic acid production, an increase in the methane content was also evident (Figure 4.1.2). As a result of microbial CO_2 utilization from biogas, the methane concentration increased from 61 ± 3 % to 71 ± 2 % (Figure 4.1.2). These observations are close to the previously reported data on MES from biogas (Das and Ghangrekar, 2018). The volumetric measurement of the off-gas was not performed regularly in this study. It is important to take into account the changes in the off-gas volume during continuous experiments for the optimization and development of such processes. The batch mode operation and consistent acetic acid production for two consequent cycles suggested biocathode development with biogas feed. No microbial growth, acetic acid production or methane upgradation were observed in control MES reactors (Figure 4.1.3).

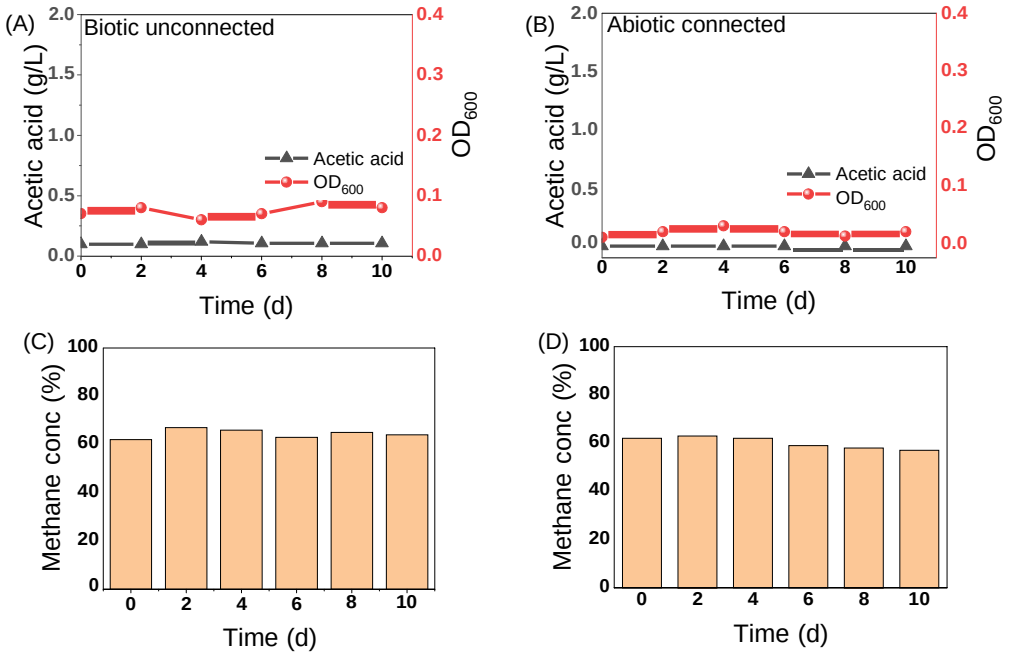

Figure 4.1.3: Acetic acid, growth (in terms of OD_{600}) and methane concentration profiles of control reactors: biotic-electrochemically unconnected (A, C) and abiotic-electrochemically connected (B, D).

4.1.3.2 MES experiments in a continuous mode operation

After developing stable performing biocathodes in a fed-batch mode operation (section 4.1.3.1), biogas was fed at different feed rates in a continuous mode. In all cases, instant microbial growth and acetic acid production were observed (Figure 4.1.4), implying successful acclimatization of the biocathode (Marshall et al., 2012). A trace amount of formic acid (up to 0.120 g/L) was also detected. As microbial growth is directly linked with organics production, the OD and acetic acid titer data correlated well (Ragsdale, 2008). Compared to the batch mode operation, the acetic acid production was considerably higher in the case of continuous feed operation.

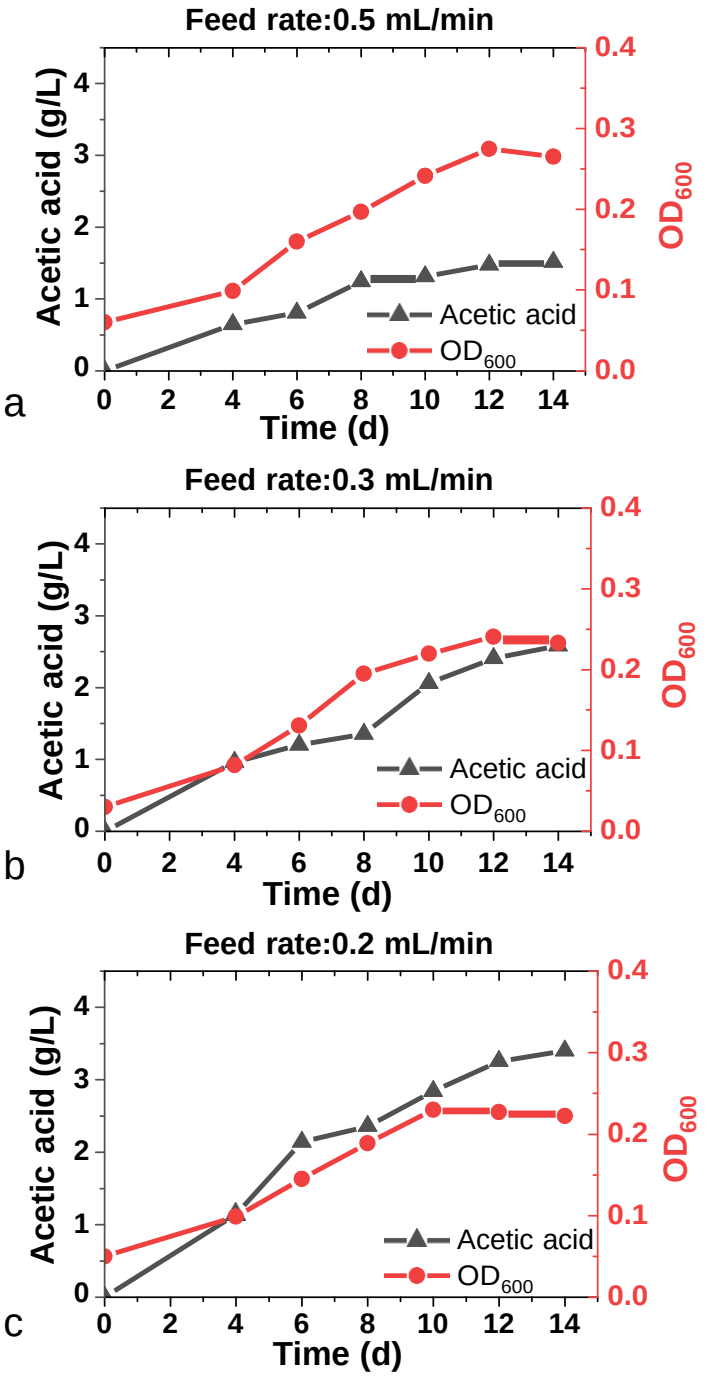

Figure 4.1.4: Acetic acid production and growth (as OD_{600}) profiles of the MES reactors fed with biogas in a continuous mode at different feed rates of 0.5 mL/min (a), 0.3 mL/min (b) and 0.2 mL/min (c).

It is mainly due to the unlimited and more CO_2 supply to the microbes in the continuous mode against a limited and less CO_2 availability in the batch mode operation. With the highest biogas feed rate of 0.5 mL/min, the maximum acetic acid titer reached 1.5 ± 0.5 g/L with a volumetric production rate of 0.107 ± 0.02 g/L/d and

a cathode surface area-based rate of 27 ± 9 g/m^2/d after 14 days (Figure 4.1.4A). The acetic acid production was leveled off after 10 days.

For 0.3 mL/min feed rate, the maximum acetic acid titer was 2.5 g/L with a production rate of 0.17 ± 0.3 g/L/d and a cathode surface area-based rate of 46 ± 7 g/m^2/d (Figure 4.1.4B). The maximum acetic acid titer of 3.4 g/L was achieved with the lowest feed rate of 0.2 mL/min (Figure 4.1.4C). It was produced at 0.23 ± 0.04 g/L/d volumetric and 60 ± 11 g/m^2/d cathode surface-based rates. The acetic acid titer tends to increase with the decrease in biogas feed rates based on a 2-fold higher titer at 0.2 mL/min feed rate than 0.5 mL/min. This can be attributed to the retention and, thereby, microbial utilization of both CO_2 and H_2 (produced at the cathode) in reactors for a longer duration at the lower feed rates.

4.1.3.3 Biogas upgradation

The methane concentration in the off-gas was analyzed at a regular time interval in a continuous mode operation. No methane upgradation was observed in the case of control MES reactors (Figure 4.1.3). Irrespective of the feed rates, an increase in methane content was prominent in all cases (Figure 4.1.5). Since microbes utilized CO_2 present in biogas as the sole carbon source, the methane content started to get enriched from the very beginning of the reactor operation at different feed rates.

At the highest feed rate, the methane concentration increased from 62 ± 3 to 82 ± 2 % on the 4^{th} day (Figure 4.1.5A) and remained within that range thereafter. A maximum methane concentration of 84 ± 2 % was observed on the 14^{th} day. The hydrogen concentration was mostly about 12 % in the off-gas. Most of the hydrogen was getting flushed out in the off-gas due to the higher feed rate. A significant increase in the methane content from 55 ± 6 to 90 ± 4 % was seen at a 0.3 mL/min feed rate after the 10^{th} day (Figure 4.1.5B). Hydrogen was mostly utilized in this particular case, and it was detected when the methane concentration was lower. The highest methane concentration was observed with the lowest feed rate after the 12^{th} day (from 62 ± 4 to 93 ± 3 %) (Figure 4.1.5C). After that, the methane concentration remained almost at the same level. The hydrogen concentration remained at a low level (< 7 %) in this case. The methane concentration enhancement was pronounced in the case of both 0.3 and 0.2 mL/min feed rates. This can be justified by the fact that at lower feed rates, microbes are able to utilize most of the carbon source for growth and acetic acid

production (section 4.1.3.2). With the goal of futuristic green fuel, a combination of hydrogen and methane can be more promising (Nanthagopal et al., 2011).

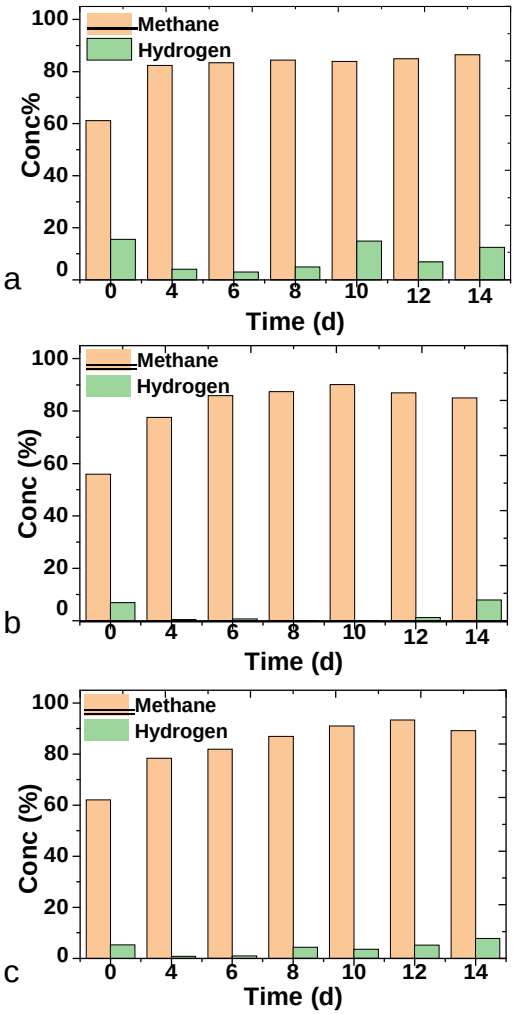

Figure 4.1.5: Methane concentration profiles in the off-gas of the MES reactors fed with biogas continuously at different feed rates of 0.5 mL/min (a), 0.3 mL/min (b) and 0.2 mL/min (c).

4.1.3.4 Electrochemical analysis of the biocathodes

In the case of control MES reactors, the reduction current was almost negligible (Figure 4.1.6) compared to that of the biotic reactors (Figure 4.1.7). A significant increase in reduction current was observed with all feed rates, and it was maximum for the lowest feed rate (Figure 4.1.7c). These observations align well with the acetic acid production data (section 4.1.3.2).

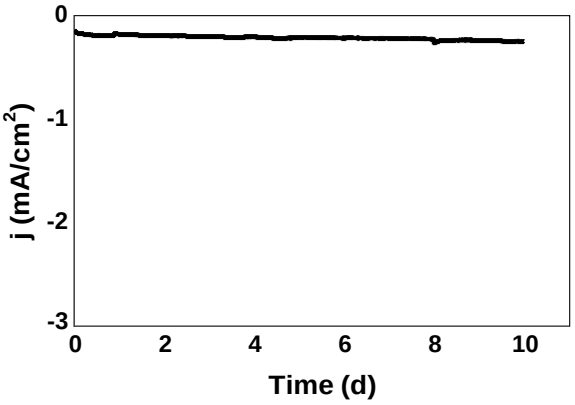

Figure 4.1.6: Chronoamperometry profile of the abiotic-electrochemically connected control MES reactor (Applied cathode potential −1 V vs. Ag/AgCl).

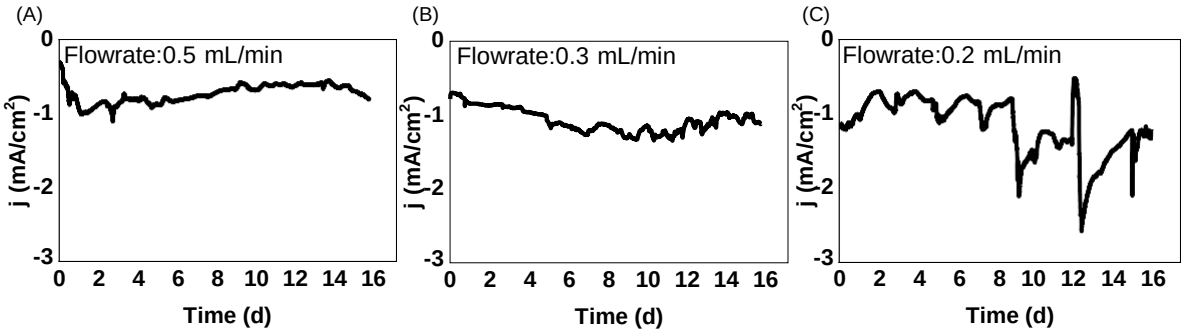

Figure 4.1.7: Representative chronoamperometry profiles at a poised cathode potential of −1 V of the MES reactors fed with biogas at different feed rates of 0.5 mL/min (A), 0.3 mL/min (B) and 0.2 mL/min (C).

Cyclic voltammograms (CVs) were recorded at different experimental conditions. The blank CV was performed before inoculation, and the inoculated and biocathode CVs were performed just after microbial source inoculation and at the end of the cycle, respectively. No redox peaks in the blank CV indicate the absence of any redox-active moieties at the cathode surface or in the catholyte of the reactors in the uninoculated condition (Figure 4.1.8). By comparing blank CV with biocathode CV, it can be seen that the H_2 evolution potential of the biocathode is higher than the bare cathode (− 0.95 V). For different feed rates of 0.5, 0.3 and 0.2 mL/min, the H_2 evolution potentials are − 0.92 V, − 0.77 V and − 0.76 V. The shift is most prominent (~ 0.2 V) in the case of the lowest feed rates (Figure 4.1.8C). This observation aligns well with the acetic acid production and methane upgradation data (Figures 4.1.4 and 4.1.5).

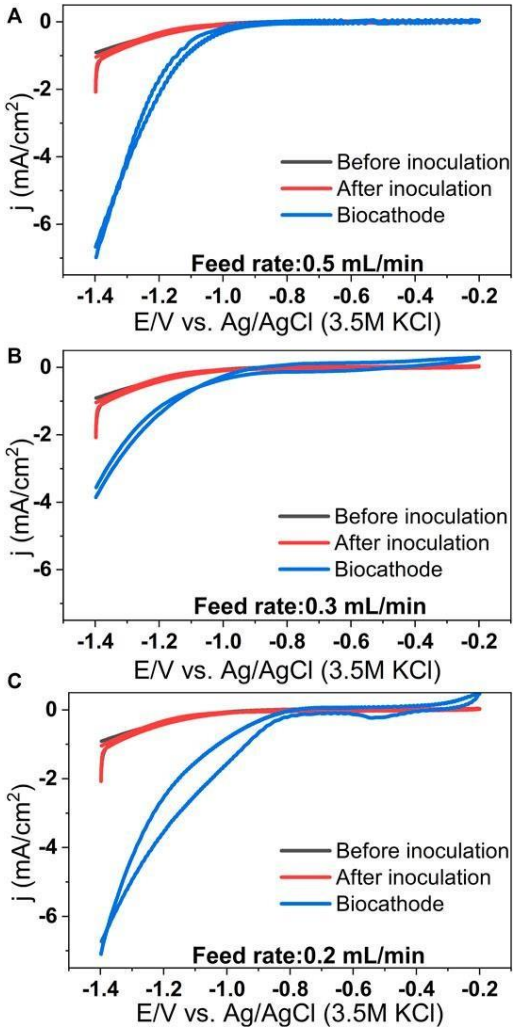

Figure 4.1.8: Representative cyclic voltammograms recorded at different conditions for the MES reactors operated at biogas feed rates of 0.5 mL/min (A), 0.3 mL/min (B) and 0.2 mL/min (C). (Scan rate: 1 mV/s).

The shift in H_2 evolution potential suggests a lowering of the H_2 evolution over-potential at the biocathode due to the presence of microbes. Moreover, the reduction current drawn at any cathode potential is higher in the case of a biocathode than the abiotic cathode due to the presence of microbial catalysts. For instance, at − 1 V, the current density (in mA/cm^2) of biocathodes are − 0.3 for 0.5 ml/min, − 0.4 for 0.3 ml/min and − 1.2 for 0.2 ml/min in comparison to − 0.08 mA/cm^2 of the bare cathode. Similar findings have been reported for CO_2-reducing biocathodes earlier (Jourdin et al., 2015a, Bajracharya et al., 2015, Patil et al., 2015a, Jiang et al., 2013). The improved electrocatalytic activity and decreased H_2 evolution overpotential can be attributed to microbial activity at the cathode of the MES systems (Jourdin et al., 2015a, Rozendal et al., 2008, Patil et al., 2015a).

4.1.3.5 Lower biogas feed rate favors efficient bioproduction and methane enrichment

4.1.3.5.1 Biogas upgradation and acetic acid production

Based on the observation of this study, it is evident that the lower biogas feed rate promotes better bioproduction and methane content increase. High feed rates ensure a non-limiting carbon supply in the reactor. At the same time, it decreases the retention time of gases in the bulk phase. As a result, the CO_2 (present in the biogas) remains for a short time in the reactor. On the other hand, though the carbon supply decreases at the lower feed rate, the retention time of gases is more in the bulk phase. In the case of higher feed rates, the H_2 produced in the cathode also gets removed readily from the system. It can influence the performance of acetogens that use H_2 as the energy source for CO_2 reduction. Hence, an optimum trade-off between the flow rate and key production parameters needs to be established through dedicated process optimization studies for the continuously fed systems. With the lower feed rate, a maximum titer of 3.4 ± 0.6 g/L was achieved, along with a methane upgradation to 93 ± 3 %. These values are comparable with the previous MES studies conducted with biogas. For example, 98 % methane upgradation was reported via electromethanogensis by Bo and coworkers in a single chamber reactor coupled with an anaerobic digester (Bo et al., 2014). Liu et al. have also reported 96 % methane upgradation by coupling electromethanogensis with an anaerobic digester (Liu et al., 2016). Even 97 % and higher methane concentrations have been achieved with a modified reactor set up (Kokkoli et al., 2018, Jin et al., 2017, Luo and Angelidaki, 2012). In terms of acetic acid titer, this study is at par with previous studies with biogas as a carbon source (Das et al., 2021).

4.1.3.5.2 Coulombic efficiency (CE)

Coulombic efficiency is the percentage of electrons recovered in the target product (Bajracharya et al., 2015, Patil et al., 2015b). A higher coulombic efficiency portrays an efficient MES system where microbes are able to utilize most of the electrons for product formation. In this study, coulombic efficiencies were quite comparable in all three conditions (Table 4.1). The highest coulombic efficiency of 90 ± 1 % in acetic acid was achieved at 0.3 mL/min along with 0.7 ± 0.03 % and 3.2 ± 1.1 % electron

recovery in H_2 and biomass respectively. With the lowest feed rate, the CE was 84 ± 7 % in acetic acid, 2.7 ± 0.6 % in biomass and 0.5 ± 0.04 % in H_2. At the high feed rate the CE achieved in acetic acid, biomass and H_2 was 82 ± 1 %, 3.7 ± 1.8 % and 1.7 ± 0.03 %, respectively. These data suggest an efficient electron recovery in acetic acid at all conditions.

Table 4.1. Comparison of key production parameters at different biogas feed rates.

Parameter	**Biogas feed rate (mL/min)**		
	0.5	**0.3**	**0.2**
Acetic acid titer (g/L)	1.5 ± 0.5	2.5 ± 0.4	3.4 ± 0.6
Production rate (g/L/d)	0.107 ± 0.02	0.17 ± 0.3	0.23 ± 0.04
Production rate ($g/m^2/d$)	27 ± 9	46 ± 7	60 ± 11
Methane enrichment (%)	62 ± 3 to 84 ± 2	55 ± 6 to 90 ± 4	62 ± 4 to 93 ± 3
Coulombic efficiency in acetic acid (%)	82 ± 1	90 ± 1	84 ± 7
Carbon recovery efficiency (%)	10 ± 4	30 ± 5	56 ± 10
Energetic efficiency in acetic acid (%)	34 ± 0.6	33 ± 0.04	29 ± 2
E_{cell} (V)	2.7	3.1	3.3

4.1.3.5.3 Carbon recovery efficiency (CRE)

Carbon recovery efficiency is an important parameter to estimate carbon sequestration in the form of the produced organic product. In this study, CO_2 was mainly recovered in the form of acetic acid. From Table 4.1, it is clear that CRE is reversely proportional to the biogas feed rate. The maximum carbon recovery of 56 ± 10 % was obtained with the lowest feed rate, followed by 30 ± 5 % at 0.3 mL/min and 10 ± 4 % at 0.5 mL/min. Similar findings were also reported by Das and coworkers, where 20 % CRE was reported with high biogas feed rates of 3.5 mL/min (Das et al., 2020).

4.1.3.5.4 Energetic efficiency (EE)

Energetic efficiency elucidates the overall energy requirement of the MES system. It helps to determine the overall operational cost of the MES system. EE is one of the critical bottlenecks that limit the effectiveness of the MES process (Prévoteau et al.,

2020). The higher the EE, the more efficient the MES system will be in producing the product of interest. In this study, around 34 ± 0.6 %, 33 ± 0.04 %, and 29 ± 2 % energetic efficiency was achieved at 0.5, 0.3 and 0.2 mL/min biogas feed rates, respectively (Table 4.1). A similar range (35-42 %) has been observed in previous MES studies but with pure CO_2 feed (Labelle and May, 2017).

When comparing the overall efficiencies, it is clear that though a lower feed rate of 0.2mL/min leads to better CRE, in terms of CE and EE, higher feed rates outperformed the lower feed rate. These observations suggest a trade-off among efficiencies at different feed rates, which needs to be understood better and optimized through further research.

4.1.3.6 Microbial community data

Acetobacterium, Desulfovibrio, Sulfurospirillum, and *Lentimicrobium* genera dominated the mixed culture (Figure 4.1.9). The relative sequence abundance of different microbial genera changed according to conditions. For example, the relative abundance of *Acetobacterium* increased in the MES reactors (38 ± 3 %) compared to the inoculum source (9 %). The relative sequence abundance of *Sulfurospirillum* was prominent in the inoculum source (16 %) and bulk phase of reactors (12 ± 1 %) but less at the cathodes (3 ± 2 %). In the MES reactors, *Acetobacterium* spp. was the most dominant genus, with a relative abundance of 18 ± 2 % in the bulk phase and 19 ± 2 % on the biocathode. This genus is known as acetogens for its H_2 and CO_2 fixing abilities into acetic acid (Ragsdale, 2008). Its primary function is to fix CO_2 through the Wood-Ljungdahl pathway for energy conservation and synthesize acetyl-CoA and biomass (Labelle et al., 2020). This genus has played a likely role in acetic acid production in this study (Section 4.1.3.2). Another dominant genus *Sulfurospirillum,* is microaerophilic, which means that it can scavenge small amounts of O_2 that may get transferred from the anode to the cathode chamber. Thus, anaerobic conditions in the MES reactors can be maintained (Goris et al., 2014). As they can oxidize acetic acid using O_2 as the electron acceptor, the acetic acid titer may decrease slightly (Labelle et al., 2020). As there are more chances of O_2 presence in the bulk phase, its abundance is higher in the bulk phase compared to the cathode.

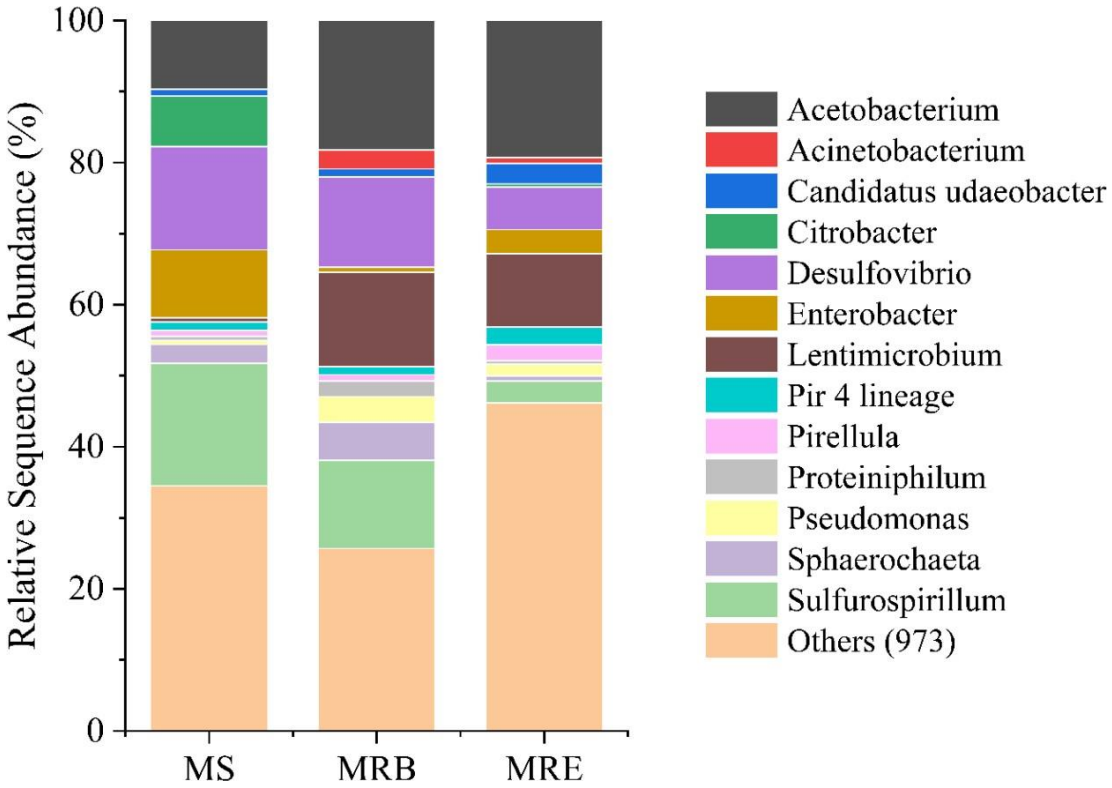

Figure 4.1.9: 16S rRNA sequencing-based microbial community composition at the genus level in the CO_2 fixing microbial inoculum source (MS) used in microbial electrosynthesis (MES) experiments, along with the MES biocathode (MRE) and MES bulk phase (MRB).

Desulfovibrio was one of the dominant genera in this study, with a relative abundance of 14 %, 12 ± 1 %, and 6 ± 2 % at the inoculum source, bulk phase, and biocathode, respectively. These sulfate reducers are capable of direct electron transfer (Aulenta et al., 2012). They can also facilitate H_2 and formate production with the help of cytochromes, hydrogenases, and formate dehydrogenase (Labelle et al., 2020). These can then be utilized as an energy source by the acetogens present in the MES system. *Lentimicrobium* genus was found to be dominant in the MES reactors. In the inoculum source, its relative abundance was only 0.5 %. Its relative abundance increased to 13 ± 1 % and 10 ± 1 % in the MES reactor bulk phase and biocathode, respectively. It is a genus of strict anaerobic bacterium previously reported in biogas upgradation studies (González-Cortés et al., 2021). It has also been reported for acetic acid production under certain conditions (Sun et al., 2016). Apart from these genera, *Pseudomonas* was also present at a low relative abundance of up to ~ 3.5 ± 0.5 % in the bulk phase and ~ 1.5 ± 1 % at the biocathode. It is known for hydrogen production

via electron transfer through redox mediators as well as hydrogenase enzymes and has been reported in bioelectrochemical reactors (Mateos et al. 2019). Some other bacterial genera, namely, *Pirellula, Sphaerochaeta* and *Enterobacter*, were also observed in very low abundance, whose role is not very clear in MES systems. Since, a methanogen inhibitor (2-bromoethanesulfonate) was added in the catholyte, no methanogens were observed in the developed microbial community over the experimental period.

4.1.4 Conclusions

This study demonstrates that efficient biogas upgradation through CO_2 conversion into acetic acid can be achieved via MES. The lowest biogas feed rates performed best in terms of methane upgradation as well as acetic acid production. A considerable decrease in H_2 evolution potential and an increase in reduction current suggested the role of microbes in the electrocatalysis process. *Acetobacterium* spp. and *Desulfovibrio* spp. dominated both the bulk phase as well as biocathode. The demonstrated biogas upgradation approach offers the advantage of obtaining two products in different phases: one in the bulk phase (acetic acid) and the other in the off-gas (methane). It takes us one step forward in developing the MES process for onsite biogas upgradation. These results encouraged further evaluation of the scalability of the demonstrated process, as presented in the next section.

4.2: Assessment of the scale-up and techno-economic feasibility of the microbial electrosynthesis process for biogas upgradation*

*The contents of this sub-chapter are adapted with permission from the following peer-reviewed journal publication:

Roy M., Saich M., Patil S. A. Scalability of microbial electro-acetogenesis process for biogas upgradation: performance and techno-economic assessment of a Litre-scale system. Energy & Fuels, 2023, 37(20):15822-15831. doi.org/10.1021/acs.energyfuels.3c02312.

4.2.1 Introduction

The H-shaped reactor design used in the earlier proof-of-concept study and commonly for MES experiments is not considered feasible for scale-up due to several limitations (Liu et al., 2023). For instance, a large distance between the cathode and anode electrodes leads to a higher internal resistance, affecting the input cell voltage. The electrode surface area to electrolyte ratio and membrane surface area to electrolyte ratio ratios are also remarkably low in this type of reactor (Liu et al., 2023). Moreover, the H-shaped reactor has a mixing dead zone near the membrane assembly. Considering long-term practical applications, such designs are also tricky to assemble and handle and are prone to leakage issues. Most of the issues mentioned above can be addressed in the parallel-plate, double-chamber reactor design.

For instance, the distance between the cathode and anode electrodes was reduced considerably from 110 mm in the H-shaped reactor to 60 mm in the parallel plate reactor in the present study. The design also led to a twofold increase in the electrode-to-volume (from 1:25 in the case of the H-shaped reactor to 1:12 for the parallel plate reactor) and membrane surface area-to-volume (from 1:3.3 in the case of the H-shaped reactor to 1:1.46 for the parallel plate reactor) ratios. A tube-type sparger and a mesh plate were introduced just above the tube sparger to ensure better distribution, mixing, and availability of the fed biogas throughout the cathode chamber (Figure 4.2.1). As a result, no mixing dead zone prevailed in the reactor. The parallel- late design is also easier to assemble and handle than the H-type design. All of these improvements and advantages make it a more appealing and scalable reactor design for MES processes. Such designs, however, need to be tested for the intended applications to understand the feasibility of their further large-scale practical implementation. To this end, this study aimed to assess the performance of the customized parallel-plate bioelectrochemical reactor (4.3 L) for biogas upgradation through the microbial electro-acetogenesis process to validate the technology further and assess its scale-up potential. The reactors were operated under a galvanostatic mode of electrochemical control at different applied current densities and biogas feed rates.

A primary techno-economic analysis was conducted considering the results obtained in this study and the maximum or target acetic acid titer achieved by MES to assess the practical feasibility of this process.

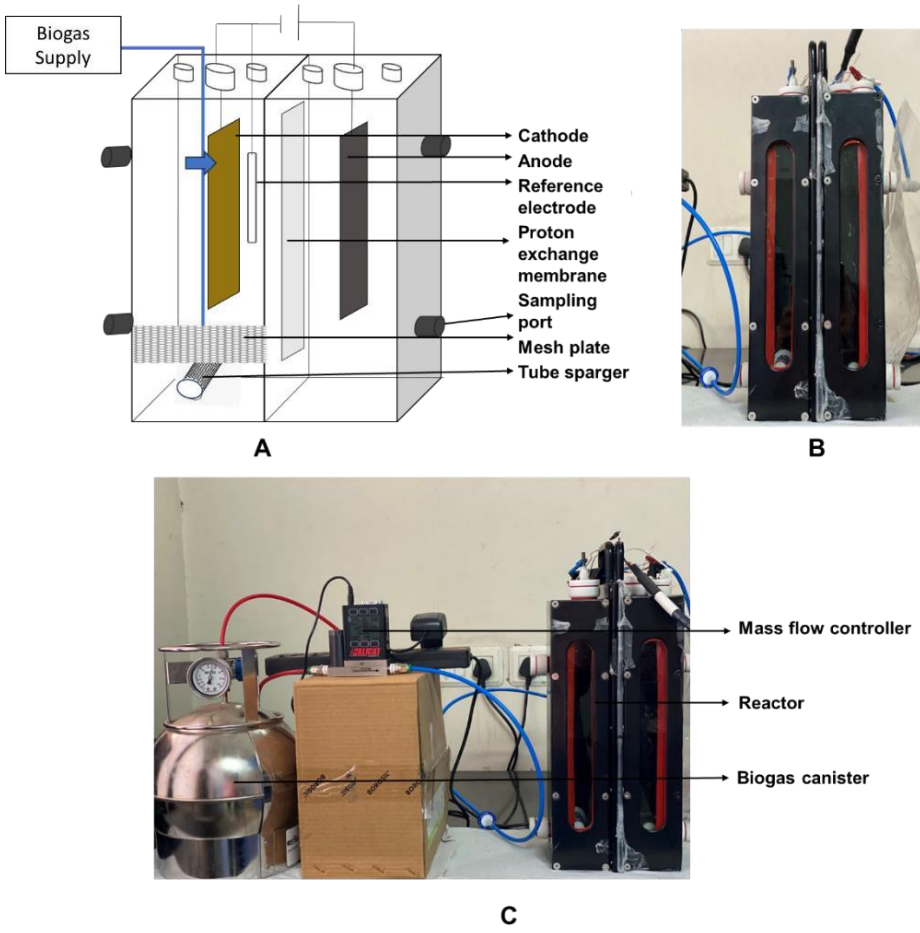

Figure 4.2.1: Schematic (A) and digital (B) images of the custom-made two-chambered microbial electrosynthesis (MES) reactor. A photographic image of the complete set up.

4.2.2 Methodology

4.2.2.1 Biogas sampling and characterization

The biogas was produced and collected from in-house operated biogas plants fed with food waste in the laboratory. Biogas was collected with the help of a vacuum pump (Model N86 KT.45.18; KNF pump) followed by storage in 15 L passivated steel canisters (SilcoCan Canister, 15 L, with 3-PortSiltek-Treated RAVE with Gauge, Restek, United States). Teflon tubing was utilized for biogas collection due to its ability to withstand high temperatures (>180 °C) and pressures (>40 psi). A 0.2 µm

PTFE filter was placed at the front of the tubing to prevent particulate matter from entering the canister. In order to reduce moisture content, a moisture trap consisted of an acrylic cartridge in which magnesium perchlorate (≥98.0 % pure, product no: 63095, Sigma Aldrich) was packed in between two layers of cotton. Further, to restrict any particulate matter and traces of magnesium perchlorate from entering the biogas canisters, a double layer of millipore filters (0.22 µm pore size) was placed at both ends of the cartridge. The composition of biogas samples was determined using gas chromatography (GC) with a thermal conductivity detector (TCD) (Agilent 490 Micro GC). The main constituents of the biogas were CH_4 (60 ± 7 %), CO_2 (37 ± 7 %), H_2 (< 1 %), H_2S (~ 2 %) and N_2 (~ 2 %).

4.2.2.2 Biogas upgradation experiments

4.2.2.2.1. Experimental setup and components

The experiments were conducted in duplicate. The overall experimental setup, along with the key reactions, is shown in figure 4.2.2. Each set-up consisted of an MES reactor to which raw biogas was fed from a gas canister via a mass flow controller (MFC). The biogas flow rate to each reactor was regulated with MFC (Alicat Scientific /MCM-20SCCMD). The MFC calibration was carried out with the air by the manufacturer. After the upgradation process in the MES reactor, the off-gas was sent to a gas displacement assembly which maintained the stability of the pressure within the reactor and helped determine the amount of biogas upgraded in the reactor. A custom-built 4.3 L capacity two-chambered reactor (2.15 L empty bed volume of each chamber), made of stainless steel with Teflon coating, was used for the MES experiments (Figure 4.2.1). The working volume of each reactor chamber was 1.8 L. Multiple sampling ports were provided in the reactor for liquid and gas sampling. The reactor design was made to minimize the distance between the anode and cathode electrode to 60 mm. It was done to lessen the ohmic resistance in the reactor and increase productivity. The cathode chamber was equipped with a tube-type sparger at the bottom position. The sparger aided in mass transfer limitation and ensured better mixing of components throughout the chamber. A mesh plate was inserted just above the sparger to ensure the availability of fed biogas to the suspended microbes throughout the cathode chamber.

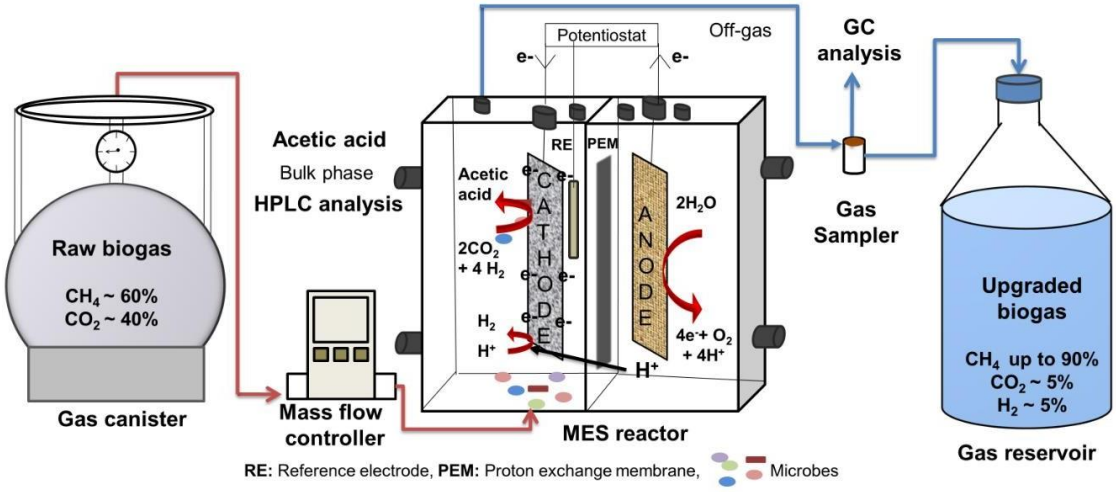

Figure 4.2.2: Schematic representation of the biogas upgradation experimental set-up and microbial electrosynthesis reactor. From left to right, the raw biogas collecting canister was connected to a mass flow controller through which the biogas was directly fed to the cathode chamber of the reactor. The upgraded biogas (off-gas) was then collected and stored in the gas reservoir.

The biogas upgradation experiments were carried out by operating MES reactors in a three-electrode configuration mode. The working electrode (cathode) was a graphite plate with a projected surface area of 150 cm^2, and the counter electrode (anode) was a dimensionally stable mixed metal (especially Ruthenium) oxide-coated titanium plate with a projected surface area of 140 cm^2. A 3.5M KCl Ag/AgCl reference was placed in the cathode chamber and close to the cathode. A 117 Nafion proton exchange membrane (PEM) separated the cathodic and anodic chambers. The graphite plate was pre-treated by acid-alkali treatment to ensure no impurities on its surface. Pre-treatment of the PEM was conducted, as explained elsewhere (Roy et al., 2021a). The catholyte was a minimal growth medium with a pH of 7. It comprised K_2HPO_4 (5.35 g/L), KH_2PO_4 (2.62 g/L), NH_4Cl (0.25 g/L), KCl (0.5 g/L), $CaCl_2{\cdot}2H_2O$ (0.15 g/L), $MgCl_2{\cdot}2H_2O$ (0.6 g/L), 1 mL/L trace metal solution, 1 mL/L selenium-tungstate solution, 2.5 mL/L vitamin solution, 2-bromoethanesulfonate (2 g/L), $Na_2S{\cdot}9H_2O$ (0.1 g/L), and 0.5 mL/L resazurin (from a 0.1 % stock solution) (Roy et al., 2021a). The cathode chamber was inoculated with a microbial culture capable of CO_2 fixation to form acetic acid. The anolyte was 0.5 M Na_2SO_4 with a pH of 2.5 (adjusted with 1 M H_2SO_4).

The anode chamber was abiotic, where the water oxidation reaction occurred ($2H_2O = 4H^+ + O_2 + 4e^-$; E = 0.82 V vs. SHE, pH 7). The H^+ ions diffuse to the cathode chamber via PEM. These protons get reduced to H_2 at the cathode ($4H^+ + 4e^- = 4H_2$; E = – 0.41 V vs SHE; pH 7). The microbial CO_2 reduction takes place in the cathode chamber where the microbes can either uptake e^- from the cathode (Direct electron transfer; $2CO_2 + 8e^- + 8H^+ \rightarrow 2CH_3COOH$)) or utilize the evolved H_2 at the cathode (H_2-mediated electron transfer; $2CO_2 + 4H_2 \rightarrow 2CH_3COOH$) to reduce CO_2 to acetic acid.

4.2.2.2.2 Microbial inoculum source

A previously enriched mixed microbial culture, dominated by *Acetobacterium* spp. and capable of CO_2 fixation to primarily acetic acid, was utilized as the source of inoculum (Roy et al., 2021a). The culture was cultivated and maintained under anaerobic conditions at a temperature of 28 ± 2 °C, under a CO_2:H_2 atmosphere (4:1), with CO_2 as the sole carbon source and H_2 as the sole energy source in a minimal growth medium. An active culture with an optical density of 0.25 (at 600 nm; OD_{600}) was inoculated in the cathode chamber of the MES reactors at the start of the experiment.

4.2.2.2.3. Microbial electrosynthesis experiments

The reactors were operated in galvanostatic mode using a Potentiostat/Galvanostat (VSP300, BioLogic Science Instruments, France). A fixed electric current was set at the cathode through the chronopotentiometry technique, and electric potential developed over the electrodes was recorded at a fixed time interval of 5 mins. The galvanostatic mode of electrochemical control was chosen in order to ensure continuous water electrolysis in the system and unlimited energy supply and improve the scalability as this mode of operation has reduced susceptibility to localized variations such as pH levels and can precisely adjust the electron flow to match the CO_2 supply rate in a stoichiometric manner (Labelle et al., 2017, Molenaar et al., 2017, Rovira-Alsina et al., 2022). The reactor was operated at different current densities selected based on the cyclic voltammetry profile of the abiotic cathode recorded before microbial inoculation of the reactors (Figure 4.2.3). Cyclic voltammetric analysis was performed at the cathode of an abiotic reactor within a potential window of – 1.2 and – 0.2 V at a constant scan rate of 1 mV/s to identify the

hydrogen evolution potential and reduction current at the specific cathode potentials. It suggests that no redox-active moiety was present in the cathode chamber (cathode and in the growth medium) before the microbial inoculation. It is clear that hydrogen evolution started at a reduction potential of – 0.975 V vs Ag/AgCl (– 0.133 mA/cm^2).

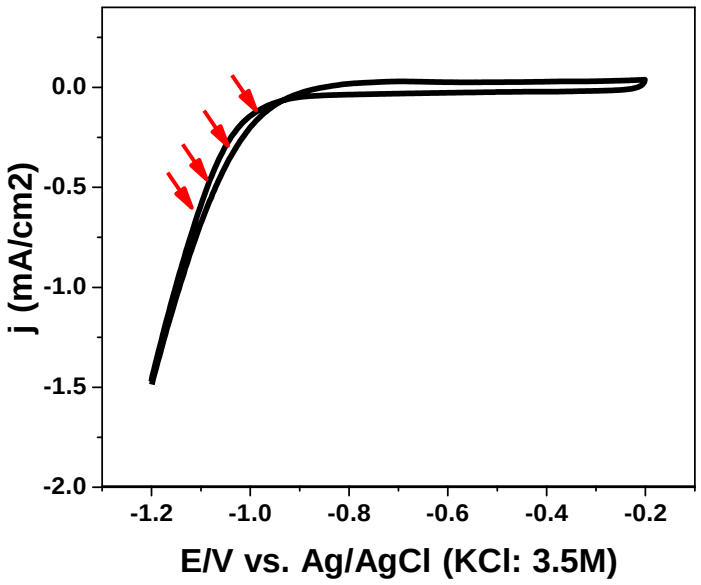

Figure 4.2.3: Representative cyclic voltammograms (CV) recorded at the cathode of an abiotic-electrochemically connected control reactor (Scan rate: 1 mV/s). The applied current densities of – 0.13, – 0.33, – 0.5, and – 0.66 mA/cm^2 are marked with red arrows.

Initially, the reactor was operated at a reduction current density of – 0.133 mA/cm^2. However, this reduction current was too high, as no H_2 production was observed. Therefore, no acetic acid production occurred in this case. So, the reduction current density was decreased to – 0.33 mA/cm^2, and the microbial community was acclimatized under these conditions. After acclimatization, the reduction current density varied from – 0.33, – 0.5, to – 0.66 mA/cm^2. These different reduction current densities were tested to determine the optimal reduction current for efficient H_2 utilization in the cathode chamber. The raw biogas was fed to the cathode chamber at a feed rate of 1 L/d at these different applied reduction currents. It also aided in maintaining anaerobic conditions in the cathode chamber. An additional experiment was conducted at the increased biogas feed rate of 2 L/d in the reactors operated at – 0.66 mA/cm^2. The upgraded biogas, i.e., the methane-rich off-gas from the MES reactors, was further sent to a gas displacement assembly, which maintained the

pressure within the reactor stable and helped determine the amount of biogas upgraded in the reactor.

Two control experiments were conducted to confirm the electricity-driven microbial CO_2 utilization from biogas in the main experiments. These include the biotic open circuit experiment (with all medium components and a carbon source but no electrical connection, i.e., no electron/energy source) and the abiotic -electrochemically connected control (with all components and an electrical connection but no microbial inoculum) experiment.

4.2.2.3 Analysis, calculations and data presentation

The cathode chamber was sampled through sampling ports sealed with a butyl rubber stopper for gas and liquid analysis at regular intervals. The off-gas composition was analysed using GC-TCD (Agilent 490 Micro GC) equipped with three channels for various gases as described elsewhere (Roy et al., 2021a). To assess the effectiveness of microorganisms in producing organics from CO_2 in biogas, bulk phase organics (C1-C4 organic acids and alcohols) were quantified using HPLC (Agilent 1260 Infinity II, RID Detector, Hiplex H column, 5μM H_2SO_4 as mobile phase, flow rate 0.5 ml/min, temperature 50 °C). In addition, OD measurements at 600 nm (Photo-lab 7600 UV-VIS spectrophotometer) and pH (Oakton PC2700) of the bulk phase were analysed to check microbial growth and pH changes in the bulk phase. The key production parameters were calculated, including product titer, production rates (normalized by catholyte volume), coulombic efficiency (CE: electron recovery into organic products), carbon recovery efficiency (CRE) and energetic efficiency (EE) considering the organic acid produced from CO_2 based on the day when the production started to level off, and maximum acetic acid titer was achieved (Das and Ghangrekar, 2018, Patil et al., 2015b). Methane data was not considered for CE and EE estimation since it was not directly associated with energy consumption in this study. The numerical data are presented as an average of two reactors with standard deviation for each tested condition.

4.2.2.4 Techno-economic analysis of the biogas upgradation process

In order to understand the economic feasibility of the process, the revenue percentage in acetic acid and upgraded methane was calculated (Jourdin et al., 2020). To compare the

performance and to understand the commercialization prospects of this process, sensitivity analysis was performed where the acetic acid titer and cell potential were varied within a specific range (Wood et al., 2021). The acetic acid titer achieved in this study was used as baseline data, and the maximum acetic acid titer of 50 g/L was considered since it is desired for downstream processes (Prévoteau et al., 2020). A gradual increase of 5 g/L titer was considered. Recently, a study on MES reported the highest acetic acid titer of 34.5 g/L at an E_{cell} of 4 V (Cui et al., 2023). Therefore, the cell potential was varied from 2 to 4 V in the sensitivity analysis. The pricing of all the chemicals was based on Indian tariffs. The cost of electricity input was estimated as per the solar energy tariff in India between Rs 2.50-2.87 per kilowatt hour (kWh). The cost calculation was based on the input and output parameters, as described in Table 4.2.1. The revenue was calculated considering upgraded methane and acetic acid products.

Table 4.2.1: MES reactor components used for techno-economic assessment (A currency exchange rate considered for INR to EURO conversion; 1 €= Rs 90)

Key variables		**Price in Euro**
MES assets / Capital cost	Reactor frame	2228.89
	Anode	16.67
	Cathode	15.33
	Wire	0.28
	Membrane	66.67
MES inputs/ Operational cost	Electricity price (€ kWh^{-1})	0.0278
	Phosphorous (€ ton^{-1}) - Triple superphosphate	0.0263
	Nutrient cost (€ L^{-1})	0.0202
	Water cost (€ kilo L^{-1})	0.075
Product revenue (output)	Acetic acid (€ ton^{-1})	650
	CH_4 (€ ton^{-1})	777

The contributors to the capital cost are listed in Table 4.2.1 and Figure 4.2.10. The breakeven point was calculated based on capital cost (considering acrylic sheet as reactor frame material and Ultrex as proton exchange membrane) and the calculated profit (with a minimum profit cutoff of 55 % based on the operational cost). It denotes the time period needed for the technology to be able to pay back its capital investment and begin generating income. The breakeven time was calculated from the point where the process starts to make at least more than 55 % of profit based on Figure

4.2.10a. The generated O_2 in the anode chamber could be utilized for combustion or other commercial purposes. However, it was not considered in revenue calculations in this study.

4.2.3 Results and Discussion

4.2.3.1 Acclimatization of the CO_2-fixing microbial community with biogas feed

The acetic acid titer and growth increased after 2-3 days of microbial inoculation in the cathode chamber of the MES reactors. It reached a stationary phase with 0.9 ± 0.3 g/L acetic acid after 16 days (Figure 4.2.4). Acetic acid was the main organic product produced in the reactors (Figure 4.2.4); hence, the MES process is referred to as the electro-acetogenesis process. By the end of the acclimatization process, the methane concentration in the off-gas increased from 54.4 ± 6.2 % to 70.7 ± 0.4% (Figure 4.2.4). These results indicate the growth and development of a stable-performing CO_2-fixing microbial culture in the cathode chamber.

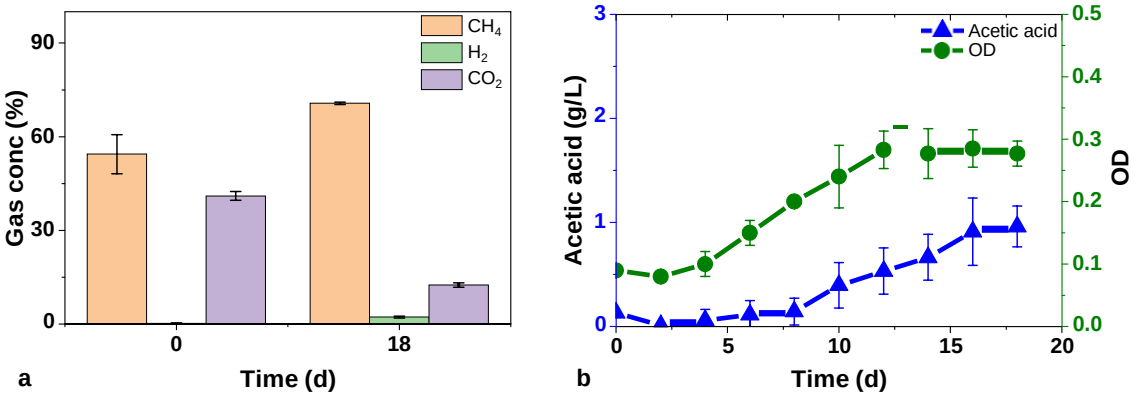

Figure 4.2.4: Off-gas (a) and acetic acid production and OD_{600} (b) profiles of the acclimatization period of the microbial electrosynthesis process with biogas feed.

4.2.3.2 Biogas upgradation

The MES experiment at each condition was run for at least two weeks to achieve a clear CO_2 utilization trend and better understand the ongoing process. Microbes were provided with CO_2 in biogas as the sole carbon source. Due to the utilization and, thus, removal of CO_2 by the microbes, an increase in CH_4 concentration was achieved in all the cases (Figure 4.2.5). At the high reduction current density of – 0.33 mA/cm^2, the CH_4 content in biogas increased from 60 ± 7 % to 88 ± 2 % after three days, and after that, it remained within that range. As high reduction current was applied in the MES reactor, H_2 was detected in low concentration (< 1 %) in the reactors, suggesting its use

by microbes for CO_2 fixation via the Wood-Ljungdahl pathway. The H_2 gas increased in the headspace at the end of the experiment when microbes could not utilize it for CO_2 fixation, and thus, organic acid production leveled off (section 4.2.3.3). With a further decrease in reduction current density to – 0.5 mA/cm^2, a remarkable increase in methane content from 56 ± 2 to 86 ± 6 % in upgraded biogas. In this case, a considerable H_2 (> 5 %) was consistently detected in the reactor off-gas. After 13 days, the CH_4 content decreased, and the H_2 concentration increased drastically. As the growth cycle of the microbial culture approached the stationary phase and the micronutrients got exhausted, microbes could not utilize the H_2 (produced in the cathode chamber) efficiently. Therefore, the decrease in CH_4 content was observed along with an increase in H_2 after 13 days. At the lower reduction current density of – 0.66 mA/cm^2, the CH_4 concentration increased from 61 ± 8 to only 75 ± 0.5 %. This low methane content in upgraded biogas was due to excess H_2 production (on average 30 %) in the cathode chamber, which was not utilized by the microbes.

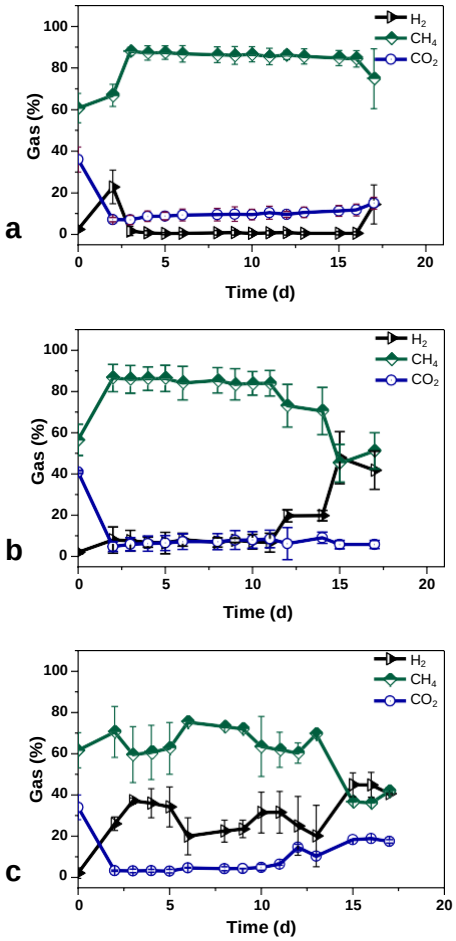

Figure 4.2.5: Off-gas or upgraded biogas profiles of the cathode chamber fed with biogas continuously at 1 L/d in MES reactors operated at different applied current densities: (a) –0.33, (b) – 0.5, and (c) – 0.66 mA/cm^2.

Therefore, the performance was not enhanced, as excess H_2 produced in the system scrubbed out the biogas before its CO_2 portion was efficiently utilized. Though the CH_4 content in upgraded biogas was less at a lower electric current condition, considerable H_2 concentration was present in the off-gas. Apart from H_2, a trace amount of H_2S (< 2 %) was also present in the off-gas, originating from the biogas feed.

4.2.3.3 Acetic acid production from CO_2 in biogas via the electro-acetogenesis process

The product titer, optical density, and change in pH in MES reactors operated at different applied current densities are shown in Figure 4.2.6a-c. Due to the resilient nature of the enriched mixed culture, which has been previously described, a rapid rise in both growth and acetic acid was observed in all instances following the initial acclimatization period (Roy et al., 2021b, Patil et al., 2015a). In the case of – 0.33 mA/cm^2, the microbes started to grow, and their OD reached 0.42 within 9 days. The acetic acid titer reached up to 1.8 ± 0.24 g/L with a production rate of 0.136 ± 0.01 g/L/d within 13 days and remained in the same range. In this case, the pH drop was not noticeable. At – 0.5 mA/cm^2 reduction current density, the acetic acid titer reached 3 ± 0.33 g/L within 12 days and after 14 days of the cycle, a maximum acetic acid titer of 3.6 ± 0.6 g/L was achieved at a 0.23 ± 0.04 g/L/d production rate. After 12 days, even when the acetic acid titer exceeded 3 g/L, a significant drop in pH was not noticeable. When this result is compared to the preceding condition, it can be elucidated that at – 0.33 mA/cm^2, H_2 might have been the limiting factor for CO_2 fixation (as H_2-mediated electron transfer plays a crucial role in this process). A similar trend was also seen in the gas data (Figure 4.2.5). Acetic acid titer did not increase further with a subsequent decrease in the reduction current. At –0.66 mA/cm^2, an acetic acid titer of 3 ± 0.5 g/L was achieved with a production rate of 0.19 ± 0.04 g/L/d. The lower applied current led to more H_2 production in this case, as reflected in the gas data (Figure 4.2.5). However, it did not increase the acetic acid production, most likely due to accelerated removal of CO_2 or limited CO_2 supply from the cathode chamber, thereby limiting its availability to the microbes. Hence, an additional experiment was conducted at an increased biogas feed rate of 2 L/d at the same current density condition (section 4.2.3.4).

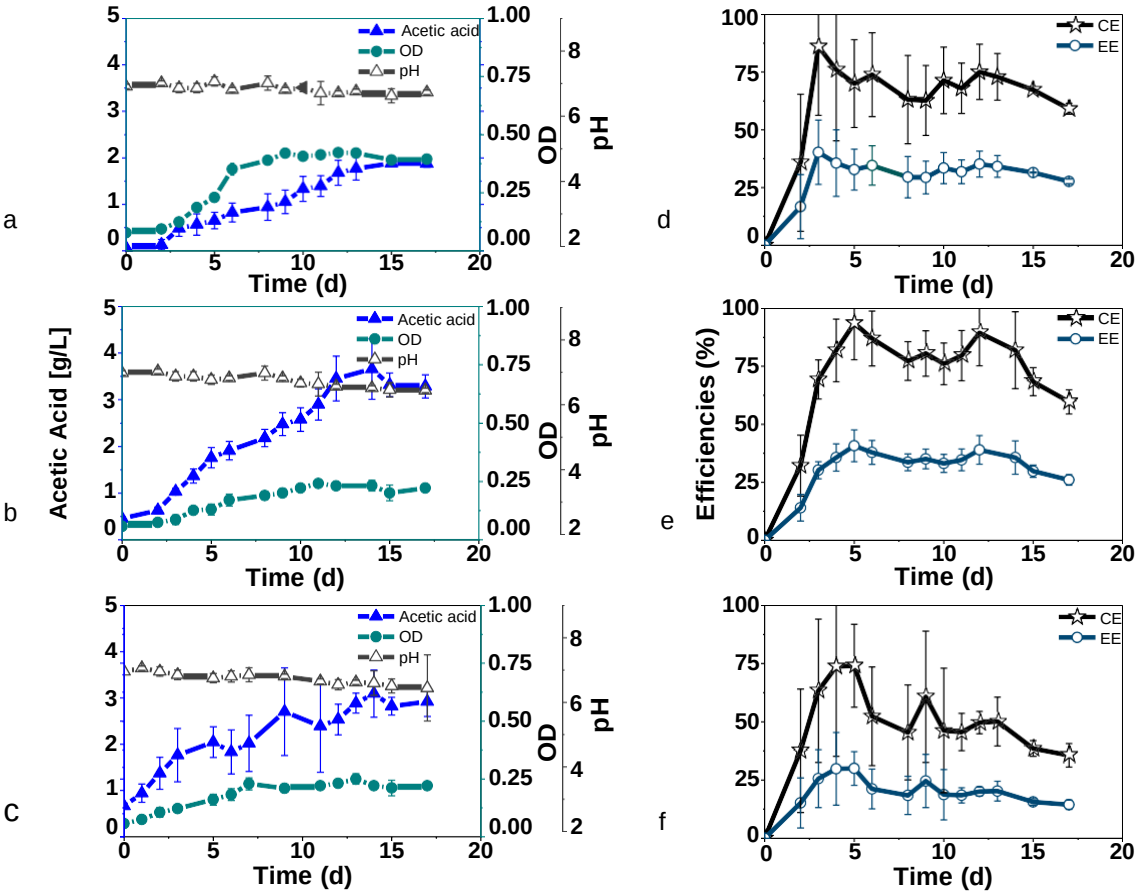

Figure 4.2.6: Growth, pH and acetic acid concentration profiles (a, b, c) along with their coulombic and energetic efficiencies (CE and EE) of the MES reactors (d, e, f) fed with biogas at 1 L/d and operated at differed applied current densities: (a, d) – 0.33, (b, e) – 0.5, and (c, f) – 0.66 mA/cm^2. The efficiencies are shown only for the acetic acid product.

Coulombic and energetic efficiencies (CE and EE) are two important parameters of MES. CE allows tracking electron recovery in the target product, which provides insight into product selectivity. The energetic efficiency (EE) reveals the overall energy requirements for producing the target product or completing the target process in the system. As CH_4 was not produced in the system, it was not considered in CE and EE calculations. These calculations are based on acetic acid as the main target product and H_2 as another electron sink. As shown in Figure 4.2.6 (d, e, f), the CE was better at the higher currents. In the case of – 0.33 mA/cm^2 and – 0.5 mA/cm^{2,} the CE in acetic acid was 73 ± 10 % and 82 ± 16 %, respectively. These data are consistent and on par with previously reported CEs attained with smaller-scale reactors (<250 mL working volume) operated at similar conditions, indicating that the larger reactor size did not impair the electron recovery or faradic efficiency of the scaled-up reactors

(Roy et al., 2021b, Das et al., 2018). At – 0.66 mA/cm^2, the average CE in acetic acid was 50 ± 10 %, and considerable electrons (39 ± 4 %) were recovered in unutilized H_2. In the case of – 0.33 mA/cm^2 and – 0.5 mA/cm^2, the CE in H_2 was comparatively much lower than that of – 0.66 mA/cm^2 applied current density condition. The EE of the process considering acetic acid as the product was 20 ± 4, 36 ± 7 and 34 ± 4.7 % at – 0.66, – 0.5 and – 0.33 mA/cm^2 conditions, respectively. Though it has been reported that the EE decreases remarkably with an increase in reactor size, these values are in a similar range to the previous proof-of-concept study on biogas upgradation in small lab-scale reactors (Roy et al., 2021b).

In the case of control reactors, neither growth nor acetic acid production was observed (Figure 4.2.7). The abiotic-electrochemically connected control data confirms that the microbial catalysts were crucial in utilizing the CO_2 of biogas. The open circuit biotic control confirms that electricity provided in the form of applied current served as the sole energy source for the microbial catalysts.

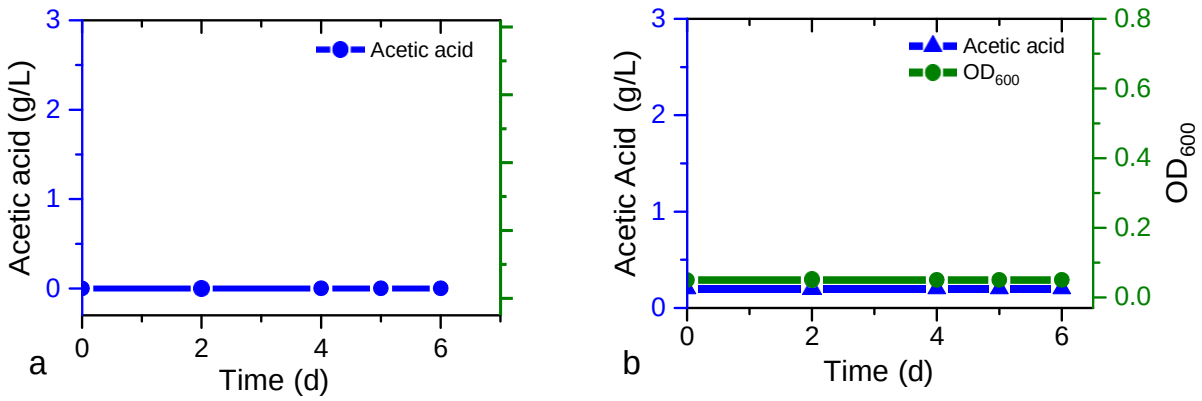

Figure 4.2.7: Acetic acid production and OD_{600} profiles of the control experiments: a) Abiotic electrochemically-connected and b) Biotic electrochemically unconnected or open circuit reactors.

4.2.3.4 Increased biogas feed rate improved bioproduction at a higher applied current

Excess H_2 was produced and underutilized in the cathode chamber fed with 1 L/d biogas at a low current density of – 0.66 mA/cm^2, so biogas was provided at a high feed rate of 2 L/d in an additional experiment. The availability of more CO_2 resulted in a better acetic acid production rate (0.33 ± 0.14 g/L/d) and titer (3.3 ± 0.14 g/L; Figure 4.2.8a) with better CE (70 ± 4 %) and EE (28 ± 1.5 %) than the low biogas feed rate condition. The upgraded biogas also had improved methane content (85 ± 7 %) and low H_2 content (18 ± 3 %) (Figure 4.2.8b) compared to the 1 L/d biogas feed

condition at the same applied current density. The effect of biogas feed rates had been investigated in a previous proof of concept study, where a trade-off among the feed rates and different production parameters was observed (Roy et al., 2021b). A similar trade-off was observed in the case of biogas feed rate and applied current density to the system in this study. A better understanding of the impact of all such operational parameters is required to fine-tune and optimize the production indicators in the scaled-up reactors.

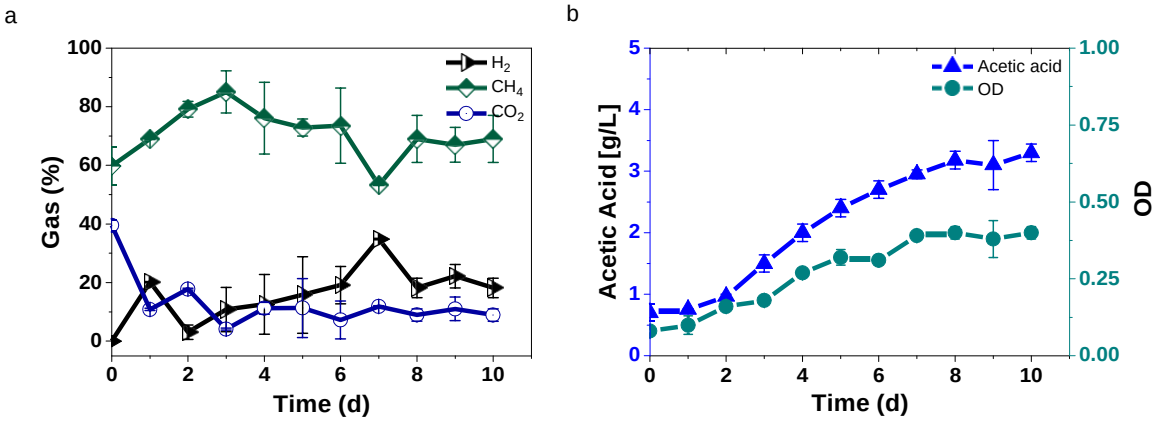

Figure 4.2.8: Off-gas or upgraded biogas profiles (a) along with the acetic acid concentration and OD_{600} data (b) of the MES reactors fed with biogas at 2 L/d and operated at – 0.66 mA/cm^2.

4.2.3.5 Performance analysis of the large-scale MES reactors for biogas upgradation

The cathode potential at different applied current densities in this study was within the – 0.8 to –1.1 V (*vs.* Ag/AgCl) range (Figure 4.2.9); hence, the H_2-based CO_2 reduction was the dominant process in the cathode chamber.

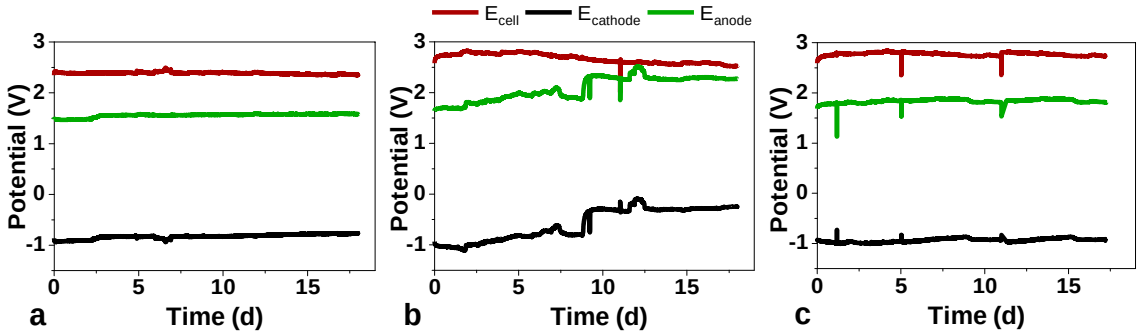

Figure 4.2.9: Potential profiles of the representative MES reactor operated in a galvanostatic mode at different applied reduction currents: (a) – 0.33, (b) – 0.5, and (c) – 0.66 mA/cm^2.

Besides acetic acid production from CO_2 and methane content increase in the upgraded biogas, the performance of the MES reactors was assessed by estimating different process parameters like carbon recovery, coulombic and energetic efficiencies, and electrochemical parameters (Table 4.2.2).

Table 4.2.2 Consolidated information on key process parameters of MES reactors fed with biogas.

Parameters	**Applied reduction current densities**			
	– 0.33mA/cm^2	**– 0.5 mA/cm^2**	**– 0.66 mA/cm^2**	**– 0.66 mA/cm^2**
	1 L/d biogas	**1 L/d biogas**	**1 L/d biogas**	**2 L/d biogas**
Max Acetic acid titer (g/L)	1.8 ± 0.24	3.65 ± 0.64	3 ± 0.5	3.3 ± 0.14
Acetic acid production rate (g/L/d)	0.136 ± 0.01	0.23 ± 0.04	0.19 ± 0.04	0.26 ± 0.01
Max Methane content increase in upgraded biogas (%)	60 ± 7 to 88 ± 2	56 ± 2 to 86 ± 6	61 ± 8 to 75 ± 0.5	59 ± 6 to 85 ± 7
Hydrogen concentration in off-gas (%)	0.5 ± 0.7	20 ± 7	44 ± 6	18 ± 3
Coulombic efficiency in acetic acid (%)	73±10	82 ± 16	50 ±10	70 ± 4
Coulombic efficiency in hydrogen (%)	0.7 ± 0.1	19 ± 2	39 ± 4	23 ± 4
Energetic efficiency in acetic acid (%)	34 ± 4.7	36 ± 7	20 ± 4.2	28 ± 1.5
Energetic efficiency in hydrogen (%)	0.4 ± 0.03	8 ± 1	16 ± 2	10 ± 2
Carbon recovery efficiency in acetic acid (%)	51 ± 7	83 ± 16	70 ± 14	49 ± 2
Ecell (V; Total cell voltage)	2.4± 0.6	2.6 ± 0.6	2.81 ± 0.55	2.8 ± 0.05

The analysis of these parameters suggests that – 0.5 mA/cm^2 current density was optimum with a 1 L/d biogas feed rate for both acetic acid production and methane content increase in biogas. The production rate (0.23 ± 0.04 g/L/d), CE (82 ± 16 %), EE (36 ± 7 %), and CRE (85 ± 16 %) for acetic acid were better with this condition. In the case of the – 0.33 mA/cm^2 current density, though the efficiencies were comparable, H_2 was a limiting factor (mostly below 1 %) for the overall process. On the other hand, excess H_2 evolution (44 ± 6 % H_2 with up to 40 % CE in off-gas) was observed in the case of – 0.66 mA/cm^2. As a result, the performance at the lower applied current was not favourable at a low biogas feed rate. At a higher biogas feed

rate of 2 L/d and a low reduction current density of – 0.66 mA/cm^2, the bioproduction of acetic acid and methane content increase in upgraded biogas was improved. However, the carbon recovery was compromised in this case since, at higher feed rates, biogas retention was affected, thereby lowering the CO_2 utilization by the microbial catalysts. These observations suggest that the right balance among the operational parameters needs to be established to maximize the production parameters without compromising the biogas upgradation process.

As observed in earlier studies with small-scale reactors, the acetic acid titer and rates remained within the same range (Roy et al., 2021b, Das et al., 2018). The production rate is an important parameter for scaling up any process. The highest production rate of 0.26 ± 0.01 g/L/d was observed at – 0.66 mA/cm^2 applied reduction current density and 2 L/d biogas feed rate. In terms of efficiencies, the coulombic and energetic efficiencies of the L-scale reactor are very much on par with the efficiencies reported for small-scale reactors and with a 50 L pilot-scale electro-methanogenesis reactor fed with pure CO_2. (Roy et al., 2021b, Enzmann and Holtmann, 2019). CE and EE at different conditions were compared, and the maximum 82 ± 16 % CE and 36 ± 7 % EE was achieved in acetic acid at – 0.5 mA/cm^2. A considerable amount of electrons (38 ± 4 %) was converted into H_2 in the case of the lower current density of – 0.66 mA/cm^2. Improved CRE was achieved in these reactors compared to the small-scale reactors. A maximum CRE of 83 % was achieved at – 0.5 mA/cm^2 condition. It can be attributed to the galvanostatic mode of operation, which stoichiometrically helps the electron flow to meet the CO_2 supply rate and improved mass transfer due to the sparger assembly in these reactors (Labelle et al., 2017, Molenaar et al., 2017, Rovira-Alsina et al., 2022). The findings of this investigation reveal that a lower cathodic reduction current (where H_2 evolution is not limited) encourages higher bioproduction of organic acids and an increase in methane concentration to a certain extent. However, with the increase in biogas feed rate to 2 L/d, the CRE decreased to 49 ± 2 % as the CO_2 portion of the biogas was not utilized efficiently at higher feed rates.

At the low reduction current, more H_2 is evolved, which is not completely utilized for acetic acid production. It leads to the mixture of CH_4 and H_2 in the off-gas. The blend of H_2 (10-30 %) with CH_4 is known as hythane, which is another potential fuel (Meena et al., 2020, An et al., 2024). It is also a desirable fuel with a net calorific value of 49.61 MJ/kg (with 30 % H_2), which is slightly better (46.28 MJ/kg) than that of CNG (compressed natural gas) with >90 % methane content. This blend may also

be utilized in an internal combustion engine without any design changes. It has a market value of 1-1.63 USD per kg (Mahant et al., 2021). Its use enhances engine performance by reducing unnecessary energy consumption. Apart from that, it can also make use of the existing natural gas system for transit and storage. Thus, the off-gas produced in lower applied current also has great potential as a fuel source.

In all cases, the process occurred at a considerable energy input. The E_{cell} profiles over time are shown in Figure 4.2.9. Initially, at – 0.33 mA/cm^2, the E_{cell} was 2.4 ± 0.6 V, but with a further decrease in the reduction current density, the E_{cell} also increased (Table 4.2.2). The maximum E_{cell} of 2.81 ± 0.55 V was observed at – 0.66 mA/cm^2 and 2 L/d biogas feed rate. In the case of the H-shaped reactor, the best-performing conditions were achieved at an E_{cell} of 3.3 V (Roy et al., 2021b). The reduced cell voltage or energy input can be attributed to the improved reactor design in terms of mass transfer limitation and reduced distance between the electrodes in the scalable parallel-plate reactors in this study. However, it is clear from the electrochemical data that the anode potential increased the total cell voltage as it was mostly >1.8 V as opposed to the cathode potential, which remained mostly within – 0.8 to – 1.1 V range in all cases. The high overpotential at the anode can be addressed by coupling other beneficial and low-energy demanding anodic reactions, thereby decreasing the overall energy input of the process (Batle-Vilanova et al., 2019, Verma et al., 2019). These include, e.g., anodic chlorine production, wastewater oxidation, and glycerol oxidation. When all the different production parameters are compared with the previously used lab-scale H-shaped reactor, the acetic acid titer, production rate, and coulombic and energetic efficiencies are within the same range as the lab-scale reactor. This indicates that the performance of the scaled-up reactor was not hampered in a few litres scale reactor. In terms of carbon recovery efficiency, it is even better than the small-scale reactor as multiple measures (for instance, tube sparging and addition of mesh plate) were taken for better gas availability in the cathode chamber. For energy consumption as well, this reactor performed better as lower E_{cell} was required (in the best performing condition) when compared to the H-shaped reactor. However, the anode potential was remarkably high. Apart from that, the production rates are still very low. These factors need to be improved to better the economic prospects of this biogas upgradation process.

4.2.3.6 Primary techno-economic outlook of the overall process

A sensitivity analysis-based techno-economic assessment was performed to check the feasibility of the biogas upgradation technology based on the operational cost of the process. It revealed that the biogas upgradation process in the Litre-scale MES system demonstrated in this study is making around 80 % loss (Figure 4.2.10a). This process can be economically feasible with a further increase in the acetic acid titer (Figure 4.2.10a). For instance, if the acetic acid titer is over 25 g/L, the profit percentage starts to increase (around 35 %), and with 50 g/L acetic acid titer and an E_{cell} of 2.6 V, around 170 % profit can be made considering only the operational cost. With the current state of the art (acetic acid: 34.5 g/L), more than 85 % profit can be achieved on the operational cost (Cui et al., 2023).

The capital cost of the set-up, including reactor fabrication, electrode materials, and membrane, was 2327 euros (INR 209505). The contribution of the different reactor items to the capital cost is shown in Figure 4.2.10b. Among the different components, reactor frame and fabrication cost were the major contributors (95.7 %) in this study, as we opted for costly Teflon-coated stainless steel (SS) as the reactor fabrication material (Figure 4.2.10b) since SS is usually preferred in large-scale bioreactors. As SS is electroactive, an additional Teflon coating was provided to make the reactor electrochemically stable. For field-scale applications, this reactor fabrication material is not suitable. There are cheaper alternatives for the reactor material, e.g., acrylic sheet, glass, polycarbonate, and polypropylene pipes. A few studies for the MES process have considered acrylic as a low-cost but durable and appropriate material for such systems (Das and Ghangrekar, 2018, Das et al., 2021, Fontmorin et al., 2021). After the reactor frame, the PEM contributed the most share to increasing the capital cost, as Nafion 117 was used in this study. The membrane cost can also be decreased using a low-cost PEM like the Ultrex CMI-7000S (Babanova et al., 2016, Jourdin et al., 2020). The cost of graphite plate cathode used in this study was lower compared to other efficient yet high-cost electrode materials. Considering an acrylic sheet for reactor fabrication and Ultrex as PEM, the reactor cost can be reduced to about 58 euros (Figure 4.2.10c). In this scenario, the capital cost gets equally distributed among the reactor frame (23 %), anode (29 %), cathode (26 %), and membrane (22 %) of the MES system.

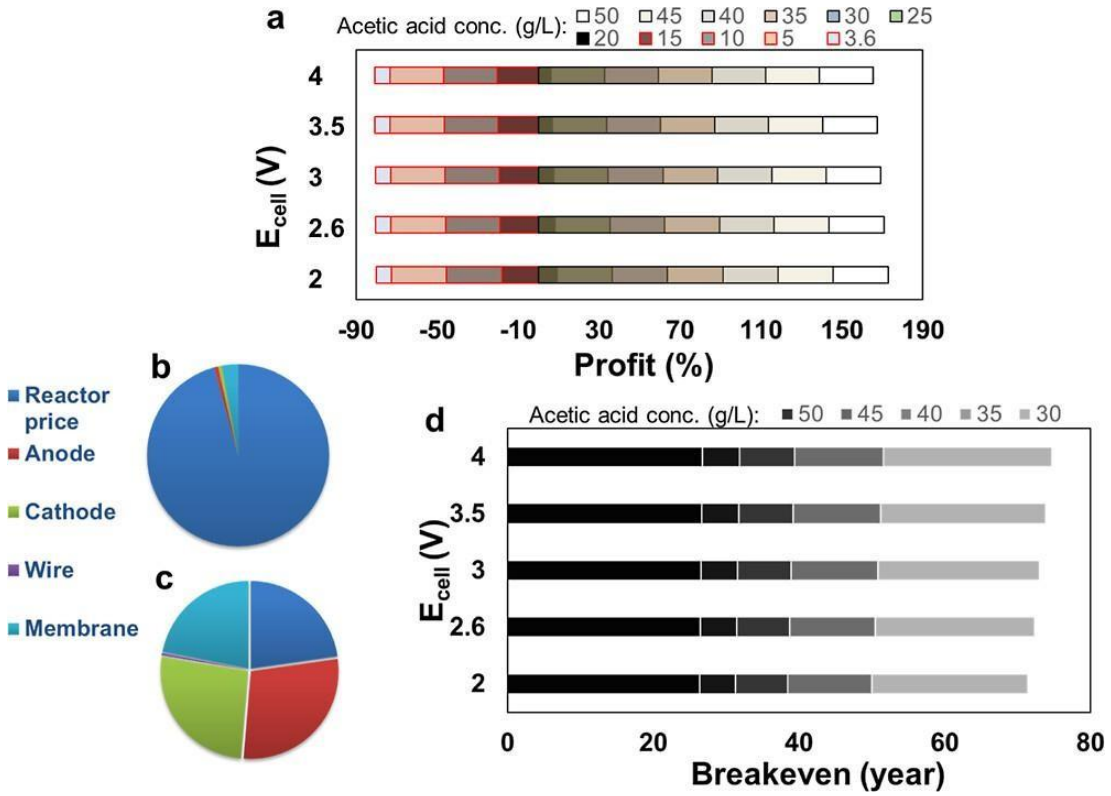

Figure 4.2.10: Techno-economic analysis of the process showing the profit % of the process at different acetic acid titers and E_{cell} (a), the key contributors to the capital cost of the reactor system used in this study (b) and an inexpensive yet efficient system made with low-cost materials (such as acrylic sheet and Ultrex PEM) (c), and the break-even point calculated based on the capital cost (d).

Considering the capital cost of this inexpensive yet efficient set up (i.e., with a reduced capital cost of 58 euros), the breakeven time was calculated for the cases where there was at least a 55 % profit margin on the operational cost. The breakeven point started after 71 years for 30 g/L acetic acid titer (Figure 4.2.10d). It can be further reduced to 26 years with an acetic acid titer of 50 g/L. The capital cost remains to be the main bottleneck to commercializing this process. The present study used commercially available efficient but costly anode and membrane materials. Low-cost but efficient PEM and anode materials should be considered for such MES processes to make the technology economically appealing.

4.2.4 Conclusions

This study successfully validated the feasibility of the microbial electro-acetogenesis or microbial electrosynthesis process for biogas upgradation in a few litre-scale

reactor. The applied current density of – 0.5 mA/cm^2 resulted in the maximum methane content increasing from 56 ± 2 % to 86 ± 6 % in upgraded biogas, along with an acetic acid titer of 3.65 ± 0.64 g/L. Besides efficient biogas upgradation and acetic acid production, the CE and EE achieved with the litre-scale reactor were on par with the routinely used small-scale laboratory reactors. These data confirm that biogas upgradation is feasible via the microbial electro-acetogenesis process even in large-size reactors without compromising on the key performance indicators. The primary techno-economic analysis based on capital and operational costs revealed that the demonstrated process is not economically viable currently and requires considerable cost savings. The high capital cost remains the major hurdle to realizing the practical application of such processes. Though this biogas upgradation process is promising, further research is required to improve the anodic reaction and production parameters of this process, besides lowering the costs associated with the anode, membranes, and other reactor components, to make its practical implementation feasible.

Chapter 5

Conclusions and Future Perspectives

This thesis sheds light on the applicability of MES technology for utilizing CO_2 from industrial sources without the need for extensive purification. In particular, MES is suitable for utilizing biogenic CO_2 derived from anaerobic digestion and fermentation processes, which are readily available yet underexplored carbon sources. It is a significant and much-needed step towards developing a sustainable and economically appealing CCU platform for utilizing unpurified industrial CO_2. The key research outcomes of this thesis work are summarized in Figure 5.1 and elaborated below.

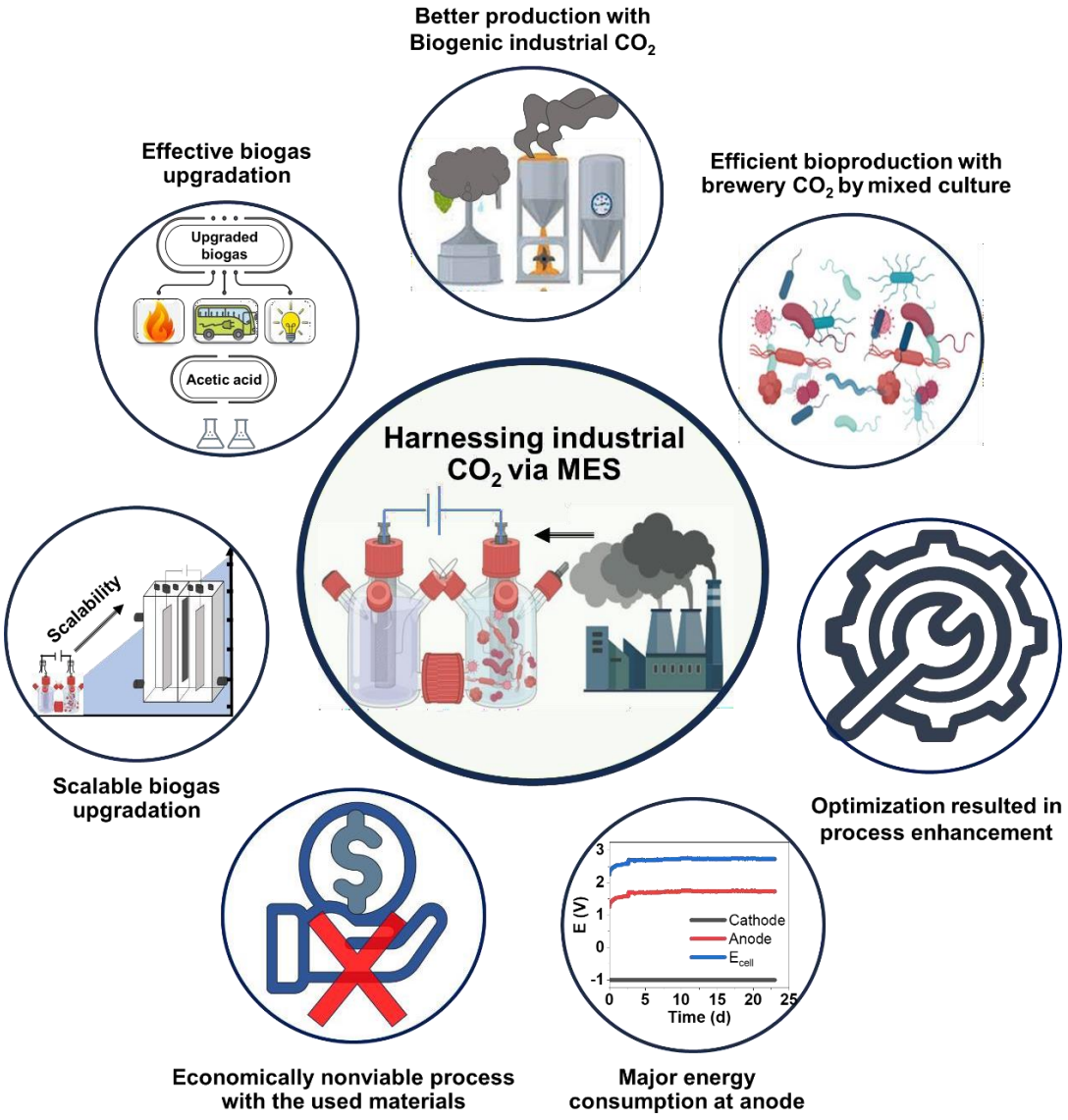

Figure 5.1: A schematic illustration of the key takeaways of the thesis work.

- The bioproduction using enriched mixed microbial culture dominated by *Acetobacterium* sp. was more promising with biogenic CO_2 sources, e.g., from the brewery and biogas plants, than other industrial sources containing more impurities. Impurities, such as NOx, SOx and O_2 are detrimental to microbial catalysts. By selectively enriching or adapting microbial communities to tolerate and metabolize the impurities present in these CO_2 streams, it is possible to develop robust microbial catalysts capable of efficient CO_2 conversion. Alternatively, additional purification or pre-treatment of these industrial gases (e.g., sugar mill, steel, and incineration plants) is required to eliminate these impurities in order to enable their utilization via bio-based CCU approaches involving anaerobic microbial catalysts.
- Utilization of unpurified brewery $\mathbf{CO_2}$ was successfully demonstrated via MES with both enriched mixed and pure *C. ljungdahlii* cultures. Due to its robustness and diverse functionality, the mixed culture exhibited superior performance to *C. ljungdahlii*. Through optimization of operational parameters and medium recirculation, acetic acid production from industrial CO_2 was further enhanced. The optimization of operational parameters and media recirculation resulted in improved acetic acid production of up to 7.6 ± 0.65 g/L at 92 % CE and an E_{cell} of 2.9 V. Further work should involve implementing larger-scale systems capable of producing not only acetic acid but also other valuable products using different microbial catalysts. Such demonstrations are crucial steps toward understanding the practical applicability of MES-based carbon utilization technologies. Future research endeavours with brewery CO_2 should aim for on-site demonstrations of scaled-up MES processes.
- MES allows effective biogas upgradation by converting CO_2 into acetic acid. The demonstrated process offers the advantage of producing two valuable products from the same biogas feedstock: methane-rich off-gas and acetic acid in the bulk phase. Biogas upgradation was feasible even in large L-scale reactors without compromising the key MES process performance indicators, suggesting process scalability. With the L-scale reactor, upgraded biogas with > 85 % methane content and acetic acid up to 3.65 ± 0.64 g/L at an E_{cell} of 2.6 ± 0.6 V were obtained. However, the process is not economically viable with the used

reactor, electrode and membrane materials based on the primary techno-economic analysis. Future studies should thus focus on reducing capital expenses by exploring alternative low-cost yet efficient reactor, electrode, and membrane materials. By identifying and implementing cost-effective materials, the overall economics of MES-based biogas upgradation can be improved, making it a more attractive and sustainable option for simultaneous carbon utilization and methane production.

- In the case of all MES experiments, enhanced electrochemical parameters, such as lowered H_2 evolution potential and increased reduction current, suggested the role of microbes in improving the electrocatalysis process. Moreover, based on the E_{anode} and E_{cell} profiles, it was evident that the anodic reaction consumed more energy in the overall MES process. Therefore, future research should prioritize investigating energy-efficient anodic reactions to enhance the overall energetic efficiency of the MES process.

In a nutshell, this thesis contributes to advancing the understanding of MES technology as one of the viable solutions for utilizing unpurified industrial CO_2 for bioproduction and underscores the importance of further research and development efforts to realize its full potential in mitigating climate change and fostering sustainable development. This work opens up the possibilities of using alternative CO_2-rich flue gases for bioproduction, providing a significant advantage over electrochemical or catalytic CCU technologies, which are prone to traces of impurities in industrial CO_2. Future research should explore developing scalable MES reactor designs and alternative low-cost but efficient reactor materials without compromising the key bioproduction process indicators. The anodic reaction of water oxidation is the major energy-consuming process in MES. Alternative anode reactions, e.g., organics oxidation, wastewater treatment, and chlorine production, should thus be investigated to make the overall process economically appealing and enable rapid development of the MES-based CCU platform.

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Chapter 3

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List of Abbreviations

%	Percentage
AD	Anaerobic digestion
CA	Chronoamperometry
CCS	Carbon capture and storage
CCU	Carbon capture and utilization
CE	Coulombic efficiency
CRE	Carbon recovery efficiency
CV	Cyclic voltammogram
CNT	Carbon nanotubes
PEM	Proton Exchange Membrane
H^+	Protons
e^-	Electrons
E_{cell}	Cell voltage
$E_{cathode}$	Cathode potential
E_{anode}	Anode potential
MES	Microbial Electrosynthesis
SEM	Scanning electron microscopy
SHE	Standard hydrogen electrode
Ag/AgCl	Silver/ silver chloride electrode
BES	Bioelectrochemical System
PTFE	Polytetrafluoroethylene
UPS	Uninterruptible power source
HPLC	High-performance liquid chromatography
RID	Refractive index detector
GC	Gas chromatography
TCD	Thermal conductivity detector
OTU	Operational taxonomic unit
EE	Energetic efficiency
CNG	Compressed Natural Gas

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