Biotransformation of carbon dioxide in bioelectrochemical systems: State of the art and future prospects

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HIGHLIGHTS

- Recent progress of bioelectrochemical CO2 reduction to products has been reviewed.
- Significant progress has been made in recent years on CO2 conversion to products.
- Most bioelectrochemical CO2 reduction approaches seem to be mediated by hydrogen.
- Future biobased CO2 refinery will integrate CO2 reduction with chainelongation.

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ABSTRACT

Carbon dioxide (CO2) utilization/recycling for the production of chemicals and gaseous/liquid energy-carriers is a way to moderate the rising CO2 in the atmosphere. One of the possible solutions for the CO2 sequestration is the electrochemical reduction of this stable molecule to useful fuel/products. Nevertheless, the surface chemistry of CO2 reduction is a challenge due to the presence of large energy barriers, requiring noticeable catalysis. The recent approach of microbial electrocatalysis of CO2 reduction has promising prospects to reduce the carbon level sustainably, taking full advantage of CO2-derived chemical commodities. We review the currently investigated bioelectrochemical approaches that could possibly be implemented to enable the handling of CO2 emissions. This review covers the most recent advances in the bioelectrochemical approaches of CO2 transformations in terms of biocatalysts development and process design. Furthermore, the extensive research on carbon fixation and conversion to different value added chemicals is reviewed. The review concludes by detailing the key challenges and future prospects that could enable economically feasible microbial electrosynthesis technology.

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1. Introduction

The escalating global energy demand as a consequence of rapid global economic growth and industrialization has led to an increase in the usage of fuels, predominantly the conventional fossil fuels
including coal, oil and natural gas, which in turn has led to high amounts of CO₂ being emitted into the atmosphere. CO₂ with its anthropogenic emissions equivalent to 52 Gt CO₂ eq/yr [1,2] is a major contributor to global greenhouse gases (GHGs) and is primarily responsible for the current climate change issues. The level of CO₂ in atmosphere is being targeted to be lowered through different approaches [3]. Two key concerted approaches that can reduce the CO₂ load in the environment are: (1) the replacement of the fossil fuels with the renewable & sustainable energy carriers and (2) the development of novel CO₂ capture and utilization technologies. One of the strategies in the second approach considers CO₂ as a C-1 building block for synthesizing fuels and various industrially relevant value-added chemicals because CO₂ is abundant, has low-toxicity and is non-flammable [4]. Although, the research targeted to CO₂ to fuels and value-added chemicals started a decade ago, none of the ‘zero-carbon’ technologies has reached commercial scale which is in part attributed to high energy requirement for the activation of CO₂ and its further reduction [5].

Currently, there are profound research efforts being made towards developing sustainable technologies to capture/fix or utilize CO₂ via chemical, electrochemical, photochemical and biotechnological routes with less energy input or by compensating the energy need by the review [5-10]. Electrochemical CO₂ reduction [8] is an effective approach for the activation and transformation of stable CO₂ molecule into fuels/chemicals such as methane [11,12], methanol [13], etc. The utilization of electricity from renewable sources for the CO₂ reduction can also alleviate the existing challenges associated with the intermittent nature of the renewable energy by storing the electricity in chemical form. Electrochemical transformation of CO₂ can be of different types; abiotic electrocatalysis which is direct electrochemical conversion, photo-electro catalysis and bio-electro catalysis. This review focuses on the bioelectrocatalytic reduction of CO₂ using whole microbes, especially chemo-lithoautotrophic bacteria, the organisms which can directly utilize CO₂ as a final electron acceptor for their metabolism. A hybrid system of biotechnology and electrochemistry, known as bioelectrochemical system (BES) has been evolved recently in this trans-disciplinary research domain. The novel BES has multiple facets of applications [14], including wastewater treatment coupled to energy generation (microbial fuel cell, MFC) [15,16], salinity removal (microbial desalination cell, MDC) [17], hydrogen production with minimum external energy input (microbial electrolysis cell, MEC) [18-20], treatment of toxic and recalcitrant pollutants (bio-electrochemical treatment, BET) [21-23], etc. Microbial electro synthesis (MES) is a currently evolving facet of BES in which CO₂ can be transformed into value added chemicals and fuels by utilizing a small amount of electrical energy in microbial catalytic system [24-26]. The excess energy generated by renewable sources like solar photovoltaics and wind turbines during the off-hours, can be effectively utilized for the transformation of CO₂ through the MES approach [24,25,27,28]. Bioelectrochemical reduction of CO₂ in MES is a specific application of autotrophic bioproduction technology which is electricity-driven, CO₂ negative and independent of biomass [25].

Several aspects of BES including microbiology, technology, and economics as well as understanding the metabolic routes involved, electron transfer mechanisms and practical considerations have been reviewed independently [25,29,30]. However, the importance of CO₂ as the feedstock for MES approach along with the facts and figures has not yet been consolidated comprehensively, which we attempted in this review. This review article focuses on the recent advancements of BES in CO₂ transformation along with the pros and cons of using CO₂ as feedstock in this system. The current status with an overview of possible fuel and chemicals synthesized and future prospects of bioelectrochemical conversion of CO₂ are comprehensively presented.

2. CO₂ availability and its utility

2.1. CO₂ availability

Industrial processes driven by the combustion of fossil fuels account for nearly 86% of global CO₂ emissions while the forestry/other land-use activities account for the rest 14% [2]. Estimates show that fossil fuels based power plants are the biggest source of CO₂ emissions, accounting for approximately 46% of total industrial CO₂ emissions [2,31]. Keeling curve (Fig. 1), which is the longest record of atmospheric CO₂ shows that the anthropogenic GHGs emissions increased more rapidly during 2000–2010 compared to previous three decades. In September 2016, the annual average global concentration of CO₂ has surpassed a record level of 400 ppm, which is widely acknowledged as a threshold with no return [1].

There are many large-volume point sources of CO₂, such as refineries and chemical industries for ammonia production, ethylene oxide production, gas processing, H₂ production, liquefied natural gas and fermentation, that emit high purity CO₂ [32,33]. However, the major contributing industries like power plants and cement industries discharge high-volume of CO₂ together with high percentages of other pollutants. The CO₂ ratio/percentage in such high volume emission remains relatively low.

Power plants are the top CO₂ emitters, discharging CO₂ as a part of flue gases (12–15% purity) at a rate of ~10.5 × 10⁶ Kt CO₂/year [34]. In terms of the quantity of CO₂ generation, the cement production industry stands at the second position after power plants, emitting CO₂ at a rate of ~0.93 × 10⁶ Kt CO₂/year with the purity of about 33%. This is followed by refineries with a production rate of ~0.8 × 10⁶ Kt CO₂/year, petrochemical industry (~0.38 × 10⁶ Kt CO₂/year), steel industry (0.65 × 10⁶ Kt CO₂/year) and other chemical industries, with different CO₂ purities. These emission quantities are alarmingly high with adverse greenhouse effects which demand an emergency in setting up new technologies for CO₂ capture at a faster rate. A synoptic comparison of the quantity of CO₂ and CO₂ concentrations in the emissions from fossil fuel combustion and biological processes based industries is shown in Fig. 2 along with the stock CO₂ in relatively perpetual atmospheric and carbonate sources. As seen from Fig. 2, CO₂ emissions from biological processes including industrial fermentation are highly pure and have high utility value in other industrial processes. If the energy input in CO₂ utilization process also is derived from renewable sources, then the process becomes independent of fossil fuels and completely CO₂ neutral conceptually.

2.2. CO₂ capture and utility

CO₂ has direct application in few industrial processes depending on its generation process and purity. The cost-efficient ways of recovering pure CO₂ from the emission sources are critical issues for the large-volume CO₂ usage. CO₂ is an inert and thermodynamically highly stable molecule with standard Gibbs free energy of formation (~394.4 kJ/mol). In addition, the concentration of CO₂ within the flue gases is also an important factor adding barrier for capturing. For example, the off-gases from power plants contains only 4–14% of CO₂, which adds more difficulty in capture and separation [37]. Major impurities present in the flue gases comprise nitrogen oxides (NOx), sulfur oxides (SOx), water vapor (H₂O), and particulate matter (PM) [31,34].

Various processes have been employed for CO₂ capture including physical adsorption [38], membrane based technologies [39], cryogenic methods [40] and hydrate formation approach [41].
Among these methods, chemisorption of CO₂ in solvents, such as monooethanolamine (MEA) has been the most effective approach in practice [42]. A major drawback of this approach is that the regeneration of the solvent from carbamate species is highly energy consuming — a unit process that accounts for up to 80% of the operating costs [38]. Besides MEA, other solvents studied for the CO₂ capture include tertiary amines, chilled ammonia and potassium carbonate. Although these solvents reduce the stripping energy, the reaction rate for CO₂ sorption in the absorber is often slow which causes difficulty in obtaining high purity carbon dioxide using a reasonable column height. The N-heterocyclic carbenes and their related complexes have also being studied as promising alternatives to achieve the effective capture, fixation, and activation of CO₂ [4].

From a sustainability perspective, biological CO₂ capture through carbonic anhydrase (CA), a naturally occurring enzyme known to catalyze the hydration of CO₂ into bicarbonate (HCO₃⁻) has been studied widely [43,44]. Extremely high turnover rate and low stripping temperature required for CO₂ regeneration make the biological CO₂ capture economically viable. CO₂ has been used in industrial production of several organic chemicals such as urea and salicylic acid, as well as in synthesis of inorganic pigments. Approximately 120 Mt CO₂ per year is utilized in industrial production: ~70 Mt CO₂/year for urea, ~30 Mt CO₂/year for organic carbonates and pigments and ~20 Mt CO₂/year for salicylic acid [6].
Other industrial applications of CO₂ include carboxylation of epoxides, refrigeration, air conditioning, fire extinguishers, packaging in food industries and as additive in beverages. Supercritical CO₂ is widely used as a processing solvent in polymer reactions such as polymer modification, formation of polymer composites, polymer blending, microcellular foaming, particle production and polymerization [45]. However, in most of these industrial processes, CO₂ is not converted into other chemicals but rather recovered per se at the end of the process. This implies that the utilization of CO₂ in aforementioned industries would lead to cumulative effects to the existing CO₂ load and perhaps is not a best solution for mitigating CO₂.

In nature, the CO₂ reduction and conversions to higher carbon molecules are accomplished by plants and other organisms via the photosynthesis, in which the energy required for the dissociation of CO₂ molecule is obtained from an external source (sunlight). Technologies that mimic the natural processes like photosynthesis are attractive in mitigating the chronic rise in the atmospheric CO₂. However, such systems, should also address the limitations of energy source, the influence of other pollutants and continuous operation efficiency without day/night variation. As chemical reactions are determined by the difference between the free energy of the reactants and the products, an additional strategy to combine CO₂ with another reactant having higher Gibbs free energy can be considered to facilitate the formation of a more convenient energy carrier [46]. Hydrogen is one such energy carrier that plays critical role in CO₂ reduction, especially in the biological systems. However, the supply of H₂ externally could make the process less sustainable and energy intensive. Bioelectrochemical processes can meet all the sustainability criteria and can answer the limitations of CO₂ reduction to multi-carbon organic compounds.

3. Basics of BES

3.1. Principles

The principle of electrochemical system lies in the fact that the coupling of oxidation and reduction reactions at two different electrodes in a single system will generate current through the circuit, which can be used directly or can be transformed into chemical energy. In principle, the electrons flow spontaneously from a low redox potential to high redox potential. For a reverse electron flow i.e. from high potential to low, an external driving force (voltage) is required. The Nernst equation gives the electrode potential of a redox reaction (Ox + n e⁻ → Red) at actual conditions as

$$E = E^\circ + \frac{RT}{nF} \ln \left( \frac{C_{Ox}}{C_{Red}} \right)$$

where $E^\circ$ is the standard reduction potential of the redox reaction at standard conditions with pH 0, R the molar gas constant (8.314 J mol⁻¹ K⁻¹), T is the temperature in K, n is the number of moles of electron transferred, F is Faraday’s constant (96485 C mol⁻¹), $C_{Ox}$ and $C_{Red}$ are the concentrations of oxidized species and reduced species at the electrode surface, respectively.

In principle, a wide range of chemical half-reactions can be used for the anodic oxidation and cathodic reduction. The cell potential ($E_{cell}$) is given by:

$$E_{cell} = E_{cat} - E_{an}$$

where $E_{cat}$ is the redox potential of cathode based on the reduction half-reaction and $E_{an}$ is the redox potential of anode based on the oxidation half-reaction. When $E_{cell} > 0$, electricity is generated whereas in the opposite case, known as electrolysis mode, external power has to be applied to run those redox reactions.

In bioelectrochemical systems microorganisms are employed to catalyze the oxidation or reduction or both reactions. Typically, microorganisms oxidize biodegradable substrates at the anode (called bioanode) to generate electric current when combined with an oxygen reduction reaction at the cathode. This technology is called as microbial fuel cell (MFC). When low potential cathode reactions, most typically the hydrogen evolution, are coupled with the oxidation of organic matter with a small external voltage application then the system is called microbial electrolysis cell (MEC).

MES is a unique application of BES for the conversion of low-value waste into fuels and chemicals at the cathode by employing electroactive microbes that are self-rejuvenating and adapting themselves to the required conversion activity[25,47]. Fig. 3 depicts the schematic representation of the three major approaches of BES, namely, MFC, MEC and MES. Microbial catalysis of electro-reduction of CO₂ to organic chemicals has been evolving as a breakthrough technology that addresses not only an alternative low-cost route for chemosynthesis, but also as a method for carbon capture and fixation [25,36,48]. Nevin et al. [24] presented the first proof of concept of MES for microbial electrocatalysis of CO₂ reduction to multi-carbon organic compounds.

3.2. Electron transfer (ET) mechanisms in BESs

Exchange of electrons between the microbes and electrodes is a unique phenomenon in BESs that links the biological and electrochemical processes. When microbes oxidize the organic matter anaerobically, the electrons generated could be transferred outside the bacterial cell directly to the electrode (direct electron transfer (DET)) or to the soluble electron acceptors (mediated electron transfer (MET)) [49]. Microbes that exchange the electrons with the external electrodes are called exoelectrogens [50] or electrochemically active bacteria (EAB) [51] and the biofilms formed are termed as electroactive biofilm. Detailed understanding on the exocellular electron transfer (EET) has been reported [21,52]. Various species of Geobacter and Shewanella are the mostly studied microorganisms in BES for EET mechanism and many other bacteria have been found to possess exoelectrogenic abilities [53].

Some electroactive bacteria have ability to receive the electrons from the cathode directly or via some redox mediators and finally reduce the electron accepting species like protons, CO₂, sulfate, nitrate etc. at the biocathode [54,55]. Unlike the EET, the exocellular electron uptake mechanism has not been fully exploited. However, the studies reported till date depict the possibility of utilizing both direct (through membrane-bound organelles) and indirect (through hydrogen) electron transfer (IET) mechanisms. Certain soluble redox mediators also facilitate the biotic and abiotic redox interactions via the EET mechanism at biocathodes [55]. Three possible mechanisms, DET, MET and IET are further detailed below.

3.2.1. Direct electron transfer mechanism (DET)

The electron exchange through DET is possible through physical contact between the electrode and microbe via outer membrane proteins, like., cytochromes etc. [56], or conductive pili (nanowires) [57,58], without the involvement of soluble redox species. Metal reducing bacteria such as Geobacter and Shewanella need solid terminal electron acceptors like Fe(III) oxides in their natural environment which is the anode in case of BESs [52]. It is also reported that Geobacter sulfurreducens and Shewanella oneidensis can
evolve electrochemically conducting molecular pili (nanowires) which provide a conductive path between the cytochromes present in their outer cell membrane and the electrode. This allows these microorganisms to reach and utilize more distant solid electron acceptors. The formation of such nanowires may allow the development of thick biofilms and thus higher anode performances [52].

3.2.2. Mediated electron transfer mechanism (MET)

The electron exchange that occurs without a physical interaction between the microbe and the electrode, mostly takes place through redox mediators, also known as electron shuttlers. These redox mediators can be self-produced, examples include flavins and phenazines [59] or externally added molecules, such as neutral red, AQDS, thionin, methyl viologen or anthraquinones, etc. [60]. Metal reducing gram negative bacterial species like Geobacter family and Shewanella putrefaciens are reported to utilize the externally introduced electron shuttles [43,61]. Addition of mediators increases the electron exchange resulting in higher current generation, but when it comes to selectivity, toxicity and stability, DET has a great advantage over MET [60].

3.2.3. Indirect electron transfer

Apart from the self-produced and artificial mediators, a few reduced metabolites like hydrogen and formate etc., produced in the electrochemical interactions, can also instigate the electron exchange. Hydrogen can be produced in the bioelectrochemical processes, by fermentative microorganisms [62] or through electrochemically at cathode (under electrolysis mode) which becomes an electron donor in the reduction reactions [63,64]. In fact, hydrogen production in BES enables a versatile microbial catalysis, but such electron exchange mechanism in bioelectrochemical processes are associated with low coulombic efficiencies, especially for electricity generation [65].

4. CO2 reduction reactions at cathode

CO2 is an extremely stable molecule under normal conditions [46] and its reduction is a non-spontaneous reaction that occurs at very negative potential [12]. However, in an electrochemical or bioelectrochemical system, this non-spontaneous reaction can be driven efficiently and with minimum energy input using appropriate (bio)catalysts [8,14]. The major products of CO2 reduction...
using aqueous electrolyte in an electrochemical process are formic acid (HCOOH)/formate (HCOO$^-$), oxalic acid (H$_2$C$_2$O$_4$)/oxalate (C$_2$O$_4$$^{2-}$), formaldehyde (CH$_2$O), methanol (CH$_3$OH), ethanol (CH$_3$CH$_2$OH), carbon monoxide (CO), methane (CH$_4$), ethylene (CH$_2$CH$_2$) and others. The half-cell reactions of CO$_2$ reduction along with their standard potentials as reported in the reviews by Hori [12], Jhong et al. [8] and Qiao et al. [66] are presented in Table 1. These reviews have provided details on the electrode, catalyst, electrolyte, products and state of the art of electrochemical CO$_2$ reduction technology.

Electrochemical CO$_2$ reduction requires specific metal catalyst and high energy input. Search for highly active, selective, and stable electrocatalysts for the reduction of CO$_2$ is still the major focus in this area. The overpotentials for electrochemical CO$_2$ reduction are normally too high, indicating that these catalysts’ are still not good enough for practical applications in terms of energy efficiency. On the contrary, the process can be made less energy consuming when biocatalysts are used. Sufficiently low electrode potentials can be created in bioelectrochemical system for CO$_2$ reduction by utilizing the biocatalysts that possess the CO$_2$ fixing metabolism.

The chemolithoautotrophic microbes — having the ability to uptake electrons from the cathode of an electrochemical cell, assist in the reduction of CO$_2$ to fuels or value added chemicals at low potentials [24,25]. The major advantage of BES comes from (i) Low energy input needed to activate CO$_2$ reduction due to the biological intervention, (ii) Selective for reactions, even on multi-step reactions, (iii) Adaptability of microbes for producing different products, (iv) Low cost design and operation, (v) Reaction at ambient conditions, (vi) Recyclability of the biocatalyst, (vii) Possibility of high value uplift in the market. It is claimed that the renewable electricity-driven bioproduction can harvest the solar energy at 100 times more efficiently than a photosynthesis derived biomass-based biofuel/biochemical production [67]. Table 2 enlists a number of biologically feasible CO$_2$ reduction reactions along with their redox potentials.

As an example of bioelectrochemical CO$_2$ reduction, production of acetate using acogenic microbes is discussed here. The reduction takes place at the cathode by accepting the electrons generated from the oxidation of water at the anode. The positive Gibbs’s free energy of reaction and negative thermodynamic cell voltage implies that reduction of CO$_2$ to acetate is a spontaneous process.

At anode (E$_{an}$), 4H$_2$O $\rightarrow$ 2O$_2$ + 8H$^+$ + 8e$^-$ $E^\circ$ = 0.82 V vs SHE at pH 7

At cathode (E$_{cat}$), 2HCO$_3^-$ + 9H$^+$ + 8e$^-$ $\rightarrow$ CH$_3$COO$^-$ + 3H$_2$O $E^\circ$ = $-0.28$ V vs SHE at pH 7

Cell voltage (E$_{cell}$) = E$_{cat}$ - E$_{an}$ = $-0.28 - 0.82 = -1.10$ V

Thus, the minimum thermodynamic voltage of $-1.1$ V vs standard hydrogen potential (SHE) must be applied. However, in reality, electrical voltage more than $-1.1$ V is required due to the significant energy losses in accompanying the reactions. The equilibrium potential of an electrochemical reaction varies with the pH of the electrolyte according to the Nernst’s equation. The influence of electrons (redox potential) and protons (pH) on an electrochemical reaction are depicted in the Pourbaix diagram which shows the region of relative predominance of the electrochemical species according to the redox potential and pH. The region of predominance for the CO$_2$ reduction to acetic acid/acetate (HAc/Ac$^-$) are shown in Fig. 4. The Pourbaix diagram shows the electrochemically stable state of CO$_2$ species based on the pH and potentials.

### Table 1

<table>
<thead>
<tr>
<th>Reduced product (solid particle)</th>
<th>Half-reaction in solution</th>
<th>Electrode potentials (V vs SHE at STP)</th>
<th>Metallic electrode Catalyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (solid particle)</td>
<td>CO$_2$ + 4H$^+$ + 4e$^-$ $\rightarrow$ C + 2H$_2$O (Acidic condition)</td>
<td>0.21</td>
<td>–</td>
</tr>
<tr>
<td>Methane</td>
<td>CO$_2$ + 2H$_2$O + 4e$^-$ $\rightarrow$ C + 4OH$^-$ (Basic Conditions)</td>
<td>–0.627</td>
<td>–</td>
</tr>
<tr>
<td>Ethanol</td>
<td>CO$_2$ + 8H$^+$ + 8e$^-$$\rightarrow$ CH$_4$ + 2H$_2$O</td>
<td>0.169</td>
<td>Cu</td>
</tr>
<tr>
<td>Ethanol</td>
<td>CO$_2$ + 6H$_2$O + 8e$^-$$\rightarrow$ CH$_4$ + 8OH$^-$</td>
<td>–0.659</td>
<td>Cu</td>
</tr>
<tr>
<td>Methanol</td>
<td>CO$_2$ + 9H$_2$O + 12e$^-$$\rightarrow$ CH$_3$CH$_2$OH + 12OH$^-$</td>
<td>–0.744</td>
<td>Cu</td>
</tr>
<tr>
<td>Ethylene</td>
<td>2CO$_2$ + 12H$^+$ + 12e$^-$$\rightarrow$ CH$_3$CH$_2$OH + 3H$_2$O</td>
<td>0.084</td>
<td>Cu</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>2CO$_2$ + 9H$_2$O + 12e$^-$$\rightarrow$ CH$_3$CH$_2$OH + 12OH$^-$</td>
<td>–0.744</td>
<td>Cu</td>
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<td>Cu</td>
</tr>
<tr>
<td>Ether</td>
<td>2CO$_2$ + 9H$_2$O + 12e$^-$$\rightarrow$ CH$_3$CH$_2$OH + 12OH$^-$</td>
<td>–0.744</td>
<td>Cu</td>
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<td>–0.744</td>
<td>Cu</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>CO$_2$ + 2H$^+$ + 4e$^-$$\rightarrow$ CO + 2H$_2$O</td>
<td>–0.106</td>
<td>Cu, Ag, Zn, Ti, Ni, Pb</td>
</tr>
<tr>
<td>Formic acid</td>
<td>CO$_2$ + 12H$^+$ + 12e$^-$$\rightarrow$ CH$_3$COO$^-$</td>
<td>0.016</td>
<td>Cu</td>
</tr>
<tr>
<td>Acetate</td>
<td>CO$_2$ + 6H$_2$O + 6e$^-$$\rightarrow$ CH$_3$COOH + 6OH$^-$</td>
<td>0.016</td>
<td>Cu</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>CO$_2$ + 4H$^+$ + 4e$^-$$\rightarrow$ CH$_3$O$_2$</td>
<td>–0.70</td>
<td>Cu, Boron doped diamond, Ru</td>
</tr>
<tr>
<td>Carbonate</td>
<td>CO$_2$ + 3H$_2$O + 4e$^-$$\rightarrow$ CO$^2_3$ + 4OH$^-$</td>
<td>–0.898</td>
<td></td>
</tr>
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</tr>
</tbody>
</table>
Thermodynamically, CO₂ reduction takes place at higher redox potentials than H₂ evolution. But when bacteria catalyze the CO₂ reduction reaction, lower potentials were applied to overcome the electrochemical resistances [69]. Since the interaction of bacteria with the electrode for the catalysis of CO₂ reduction is still not clearly known, understanding the mechanism of electron transfer in an undefined mixed culture biocathode is more complex. On the other hand, the biological pathways of CO₂ reduction to acetate under H₂ as electron donor have been known. Furthermore, H₂ can be produced electrochemically via water electrolysis at the cathode potential lower than −0.414 V vs SHE (H₂ evolution onset potential at biological condition). So, when the reduction occurs below the hydrogen evolving potential, it is considered that the bacteria are gaining electrons from the electrochemically produced H₂ [70].

Within the context of electron transfer mechanisms in CO₂ reduction process, a quick glance at Pourbaix diagram (Fig. 4) reveals that the area above the H₂ evolution and below the CO₂/HCO₃⁻ reduction line represents the region of dominance of DET for bioelectrochemical CO₂ reduction unless the reaction is said to be H₂ mediated when the redox potential and pH conditions fall in the region below the hydrogen evolution line.

### 4.1. Biocatalyst for CO₂ reduction

The efficiency of CO₂ reduction in BES is strongly influenced by the nature of biocatalyst. The electroactive biofilms with strong CO₂ reducing ability are most suitable for MES. Chemolithoautotrophic bacteria are widely studied for the CO₂ fixation using hydrogen and are also crucial for their electroactivity towards CO₂ reduction in MES. Several autotrophic bacteria such as Clostridium ljungdahlii, Clostridium acetigenum, Moorella thermoacetica, Sporomusa ovata, Sporomusa sphaeroides, Sporomusa silvacetica were reported for the successful production of acetate from electrode driven CO₂ fixation [24,71]. Many other bacterial species were identified in mixed cultures that can reduce CO₂ to organic molecules. The CO₂ reducing biocathode developed from the sediment inoculum was reported to contain Trichococcus palustris sp., Oscillibacter sp., Clostridium propionicum, Clostridium celerecrescens, Desulfotomaculum sp., Tissierella sp., and few other species [72]. In another case, the parent inoculum collected from wastewater basin of a brewing company and the MES reactor operated for long term was dominated with Acetobacterium sp. along with the prevalence of Sulfurosirrillum sps. and an unclassified Rhodobacteraceae [64]. Sulfate reducing bacteria were also abundant in biocathodes [73]. Recently, a genetic analysis was done for Clostridium ljungdahlii and this strain was genetically modified to produce butyrate from H₂ and CO₂ instead of acetate [74]. Adaptation of this kind of strains for the biocathodic applications will help to target high value commodity chemicals from CO₂ reduction. Development of stable and active biocathode (suspended or biofilm) from acetogens enrichment has been attained with significant improvements in the MES setup and electrode materials [27].

### 4.2. The role of hydrogen in microbial electrosynthesis during CO₂ reduction

Hydrogen is one of the well-known sustainable fuels that functions as a zero-emission energy-carrier, primarily used in the proton exchange membrane fuel cells (PEMFCs) and internal combustion engines [75,76]. In the context of MES, hydrogen functions as a high energy reductant for chemotrophic microbes which contain hydrogen metabolizing enzymes, reversible hydrogenase, that can oxidize molecular hydrogen and supply required energy [77,78]. In presence of excess H₂ under anaerobic conditions, certain microbes will oxidize H₂ to protons and CO₂ will act as prominent electron acceptor, resulting in the formation of products such as acetic acid (acetate at pH above 7) [24], glycerol [79] and butyrate [80]. The discovery of hydrogen initiated microbial reduction of CO₂ to acetate was reported in bacterial species Clostridium aceticum as early as 1932 by Fischer et al. [70]. Homacetogenic bacteria, Acetobacterium woodii, is known to produce acetic acid only in the presence of hydrogen and carbon dioxide [81].

Many bacterial species such as Geobacter and Sporomusa, C. ljungdahlii and M. thermoacetica, are capable of reducing CO₂ to multi-carbon organic molecules by directly accepting electrons from electrodes, if poised at suitable potential [71,79,82]. However, the electron demand for CO₂ reduction in this route is higher and in electron-deficit conditions, these microbes would adapt to the alternative metabolic pathways, such as fermentation or respiration. Addressing the larger electron demand for CO₂ reduction is one of the key operational challenges for MES, which can be balanced by providing in situ produced hydrogen or externally added hydrogen.
In certain organisms, hydrogen plays a crucial role of byproduct electron carrier in the electron transport chain. The presence of byproduct electron transport molecules is obligatory for certain species such as *A. Woodii*, as they are not capable to directly accept electrons from the electrodes [71]. Investigations indicate that *A. Woodii* can reduce CO₂ in the presence of externally supplied hydrogen and the metabolism stops as soon as the external supply of hydrogen is ceased [71].

The role of abiotically generated/externally added hydrogen during acetogenesis of CO₂ by a mixed culture containing *S. ovata* was studied in detail by Blanchet and al. [70]. The electrogenesis was performed at two potentials: −0.36 V and −0.66 V vs. SHE. Although both potentials favor the acetate production thermodynamically, the authors found only the experiment carried out at −0.66 V resulted in the reduction of CO₂. Under neutral pH, no hydrogen evolution occurred when the potential was above −0.41 V vs SHE. No acetate formation was observed at −0.36 V vs SHE. At −0.66 V, hydrogen was produced at the cathode which consequently mediated the acetate formation. Furthermore, when the same inoculum was sparged with H₂:CO₂ gas mixture using a gas-liquid contactor (GLC), it produced up to 10 times more acetate. Although the production is increased with GLC, the hydrogen conversion rate of 1.6–2.7% was significantly low as compared to the 53% conversion rate observed in the microbial electrochemical reactor (MER) without GLC. Smaller hydrogen bubbles were observed in MER and these smaller bubbles led to more efficient gas to liquid transfer.

Although H₂ sparging through gas-liquid contactor makes the microbial CO₂ reduction process more effective, its implementation in industrial scale has significant technical challenges. For example in MES for CO₂ reduction where the direct electron transfer to the biofilm was considered, the current densities reported were low to reach economically efficient conversion rates. At higher current densities on the other hand, hydrogen evolution becomes dominant which affects the biofilm stability. A medium-term strategy of the MES scale up was proposed by Blanchet et al. [70] which consists of a hybrid system where MER is connected downstream to a conventional water electrolysis system. This hybrid system would maintain high hydrogen evolution without affecting the bacterial medium composition or electrode corrosion.

5. **Products from CO₂ reduction in MES**

Though at the lab scale, MES approach has been used to produce a wide range of fuels (gaseous and liquid) and chemicals using CO₂ as a feedstock. Acetate/acetic acid has been one of the most widely reported product in MES studies and has been reviewed extensively recently [83]. Different products synthesized from CO₂ via MES are discussed further here.

5.1. **Organic acids**

5.1.1. **Acetate**

Most MES studies till date have focussed on acetate production using the enriched homoacetogens which can grow on wide range of organic molecules including simple gases, such as, CO₂/CO and H₂. Acetogens are flexible with the autotrophic and heterotrophic metabolism and they can grow in acidic, alkaline, saline or hot environments. Variation in the product yield and rates reported in different studies is due to the types of microbes used and their acquired metabolic pathway that mainly influence the chain length and functional group. Major CO₂ reducing microbial pathways include Calvin-Benson-Bassham-cycle (photosynthesis), Reductive TCA (Arnon-Buchanam cycle), Reductive Acetyl-CoA (Wood-Ljungdahl) cycle and Acyl-CoA carboxylate pathway. Wood–Ljungdahl pathway (WLP) is one of the best explained on the CO₂ fixation by autotrophic metabolism that present in many bacteria [84,85]. Several lithoautotrophs are reported for the metabolic CO₂ reduction to acetate and other multi-carbon compounds with hydrogen as an electron donor [86,87]. Although the major product of CO₂ reduction is acetate, minor products, such as alcohols and other carboxylates [27,80], or methane (CH₄ if methanogens are present) are also produced [63] when homoacetogenic bacteria catalyze the CO₂ reduction reaction.

The first proof of concept of biocathode-driven acetate production from CO₂ reduction has used *Sporomusa ovata* [24]. Mixed-cultures were also widely explored as biocatalyst in MES due to their ease of handling and synergetic growth with minimum maintenance of aseptic conditions which eventually helps to lower the operation costs [29,88]. Suppression of the methanogenic activity is one of the key requirements for higher acetate production rates. The selective enrichment of the biocatalyst from a mixed culture has shown to enhance the acetate production rate almost five-fold from 1.3 mM d⁻¹ [63] to 6.3 mM d⁻¹ [27]. Likewise, the methanogen suppressed mixed culture biocathodes were reported to attain higher volumetric acetate production rates: 17.25 mM d⁻¹ (1 g L⁻¹ d⁻¹) at −0.79 V vs Ag/AgCl [64] and 11.67 mM d⁻¹ at −1.34 V vs Ag/AgCl [89]. High production rate reported by Marshall et al. [64] can be linked to the use of granular graphite biofilm as the cathode which resulted in an abundant surface for the bacterial adhesion and effective electrode-interaction for the microbe. The volumetric acetate production rates reported by Labelle et al. [90], Patil et al. [88], Bajracharya et al. [36] with acetogens enriched biocathode for CO₂ reduction are comparable. An overview of improvements made in the process of CO₂ reduction in MES system in the recent literature is presented in Table 3. As the bacterial interaction with the electrode is associated with the electrode coverage of the bacterial biofilm, the production rates are expressed based on the surface area. However, planktonic production which does not rely on the biofilm is also possible.

In the studies of CO₂ reduction in MES with *S. ovata* biocathode, surface modifications with positively charge material, metal nanoparticles catalysts and carbon nanoparticles [91] have resulted in at least three times higher acetate production rate than the usual graphite stick electrodes at −0.6 V vs Ag/AgCl. The highest production rate of 3.38 g m⁻² d⁻¹ was reported at −0.6 V vs Ag/AgCl with Ni-coated carbon cloth biocathode of *S. ovata* for CO₂ reduction [92]. Recently, the use of 3D graphene functionalized cathodes was reported to improve the CO₂ reduction rates in MES using *S. Ovata* [93]. Tremendous improvements in the surface based acetate production rate have been reported for mixed culture biocathode in recent literature with carbon-nanotubes modified cathode surface reaching 685 ± 30 g m⁻² d⁻¹ [94] and 1330 g m⁻² d⁻¹ [95]. In the latest development of CO₂ reducing biocathode, acetate accumulation of up to 7–10 g L⁻¹ was repeatedly achieved with CO₂ reduction at −1 V vs Ag/AgCl which was a remarkable acetate production without methanogenesis [36]. Similar concentrations of acetate were achieved by Marshall et al. [64] with granular cathode and by Jourdin et al. [94] with surface modified cathode. In the mixed culture environment where multiple interfering processes were occurring simultaneously, such a high accumulation of acetate is a major achievement towards active biocatalyst development.

MES from CO₂ using pure culture biocatalyst consistently reported CE higher than 80% [24,91,92], showing minimum loss of energy and also operated at higher cathode potential (−0.4 V vs SHE). The CE reported in literature for mixed culture biocathode varies between 40 and 70%. Most of the losses can be attributed to the electrode overpotentials and also to the side reactions mainly H₂ evolution when it is not mediating the CO₂ reduction. However,
Likewise, Modestra et al. [99] reported the production of propio-
by the much negative applied potential used in the experiment.
production of butyrate along with acetate might have been caused
quantities during acetate production from carbon dioxide [71]. Co-
to produce medium chain organic acids, 2-oxo-butyrate, in small
nate up to 44 mg L\(^{-1}\) from 9.5 g L\(^{-1}\) bicarbonate in catholyte using enriched mixed culture at
0.6 V vs SHE. Butyrate production was also reported by Ganigué

5.1.2. Butyrates and other organic acids

The chain elongation of acetate by H\(_2\):CO\(_2\) produces propionate
(C\(_3\)), butyrate (C\(_4\)) and caproate (C\(_6\)) [102,103]. Thermodynamically,
CO\(_2\) or bicarbonate can be reduced to butyrate at a cathode potential of –0.37 V vs SHE under biological conditions. Bio-
electrochemical reduction of CO\(_2\) to butyrate was observed as additional product during the acetate production using aceticogenic bacteria as cathodic biocatalyst [27,80]. Bacteria such as S. sphe-
roides, C. ljungdahlii, Caecetium and M. thermoacetica were reported to
produce medium chain organic acids, 2-oxo-butyrate, in small
quantities during acetate production from carbon dioxide [71]. Co-
production of butyrate along with acetate might have been caused by
the much negative applied potential used in the experiment.
Likewise, Modestra et al. [99] reported the production of propio-
nate up to 44 mg L\(^{-1}\) and iso-butyrate up to 15 mg L\(^{-1}\) from
9.5 g L\(^{-1}\) bicarbonate in catholyte using enriched mixed culture at
0.6 V vs SHE. Butyrate production was also reported by Ganigué
et al. in MES from CO\(_2\) and stated to be related to chain elongation
[80]. Recently, in a continuous operation of MES systems controlled
at 9.3 A m\(^{-2}\) current, butyrate concentration of up to 0.59 g L\(^{-1}\) at
0.54 g L\(^{-1}\) day\(^{-1}\) production rate and 58.9% electron recovery has
been reported [104]. Besides butyric acid, poly-hydroxy-alkanoates
(bacterial bioplastic), a renewable value-added end-product, can be
synthesized in MES [105]. Even though production of C\(_4\) com-
 pounds is more complicated and less investigated than C\(_2\) com-
 pounds, one could expect that the biochemistry and microbiology
of the various bacteria involved could produce even higher mol-
ecular weight compounds, if microbial pathways will be under-
stood and optimized.

5.2. Solvents

5.2.1. Ethanol and butanol

Alcohols and medium chain fatty acids not only have higher
economic value but also be easily extracted from the medium [103]. Homoacetogens, such as C. ljungdahlii and C. autoethanogenum are
reported to reduce CO\(_2\) to ethanol along with VFAs at lower pH if

Table 3

<table>
<thead>
<tr>
<th>References</th>
<th>Microbial inoculum</th>
<th>Cathode material</th>
<th>(E_{\text{cat}}) (V vs SHE)</th>
<th>Current density (A m(^{-2}))</th>
<th>Acetate production rate [mM d(^{-1})]</th>
<th>Projected Area based acetate production (g m(^{-2}) d(^{-1}))</th>
<th>Max. acetate titer (g L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevin et al. [24]</td>
<td>Sporomusa ovata</td>
<td>graphite stick</td>
<td>–0.4</td>
<td>0.208</td>
<td>0.17</td>
<td>1.3</td>
<td>85</td>
</tr>
<tr>
<td>Nevin et al. [71]</td>
<td>Clostridium ljungdahlii</td>
<td>graphite stick</td>
<td>–0.6</td>
<td>ng</td>
<td>0.013</td>
<td>0.1</td>
<td>88 ± 2</td>
</tr>
<tr>
<td>Marshall et al. [69]</td>
<td>Brewery WW sludge</td>
<td>graphite granules and graphite rod</td>
<td>–0.59</td>
<td>ng</td>
<td>4</td>
<td>ng</td>
<td>67</td>
</tr>
<tr>
<td>Zhang et al. [91]</td>
<td>Sporomusa ovata</td>
<td>Carbon cloth chitosan CNT-cotton</td>
<td>–0.4</td>
<td>–0.475</td>
<td>ng</td>
<td>2.7</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>Nie et al. [92]</td>
<td>Sporomusa ovata</td>
<td>Ni-coated graphite stick</td>
<td>–0.4</td>
<td>–0.63</td>
<td>1.13</td>
<td>3.38</td>
<td>82 ± 14</td>
</tr>
<tr>
<td>Giddings et al. [96]</td>
<td>Sporomusa ovata</td>
<td>graphite stick</td>
<td>–0.74</td>
<td>–1.7 ± 0.19</td>
<td>4.68 ± 1.27</td>
<td>9.68 ± 2.65</td>
<td>89 ± 12</td>
</tr>
<tr>
<td>Su et al. [97]</td>
<td>Mixed culture domestic WWTP sludge</td>
<td>Carbon felt</td>
<td>–0.7</td>
<td>–2.96</td>
<td>15.67</td>
<td>10</td>
<td>89.5</td>
</tr>
<tr>
<td>Marshall et al. [64]</td>
<td>Adapted brewery WW sludge</td>
<td>carbon felt</td>
<td>–0.59</td>
<td>ng</td>
<td>17.25</td>
<td>ng</td>
<td>69</td>
</tr>
<tr>
<td>Zaybak et al. [72]</td>
<td>Adapted brewery WW sludge</td>
<td>Bog sediment</td>
<td>–0.4</td>
<td>0.03</td>
<td>0.05</td>
<td>0.063 ± 0.008</td>
<td>35.2 ± 4.4</td>
</tr>
<tr>
<td>Labelle et al. [90]</td>
<td>Adapted brewery WW sludge</td>
<td>graphite rod</td>
<td>–0.6</td>
<td>–1.2</td>
<td>3.6</td>
<td>10.8</td>
<td>40</td>
</tr>
<tr>
<td>Jourdin et al. [98]</td>
<td>Developments of WWTP sludge</td>
<td>nanoweb RVC</td>
<td>–0.85</td>
<td>–37 ± 3</td>
<td>0.42 ± 0.06</td>
<td>195 ± 30</td>
<td>78.5</td>
</tr>
<tr>
<td>Jourdin et al. [94]</td>
<td>Butyrate-biodegradable WWTP</td>
<td>RVC foam with SS wire</td>
<td>–0.85</td>
<td>–102 ± 1</td>
<td>ng</td>
<td>685 ± 30</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>Patel et al. [88]</td>
<td>Butyric acid enriched culture</td>
<td>Carbon felt</td>
<td>–1.12 ± 0.08 ± 5</td>
<td>1 ± 0.09</td>
<td>19 ± 1.7</td>
<td>58 ± 5</td>
<td>1.29 ± 0.15</td>
</tr>
<tr>
<td>Bajracharya et al. [65]</td>
<td>Butyric acid enriched culture</td>
<td>Carbon felt with SS mesh</td>
<td>–0.9</td>
<td>10</td>
<td>1.3</td>
<td>40</td>
<td>40–50</td>
</tr>
<tr>
<td>Giddemay et al. [89]</td>
<td>Butyric acid enriched culture</td>
<td>Carbon felt</td>
<td>–1.14 ± 0.04</td>
<td>ng</td>
<td>11.67</td>
<td>23.35</td>
<td>61</td>
</tr>
<tr>
<td>Rodestra et al. [99]</td>
<td>Mixfer culture</td>
<td>graphite plates</td>
<td>–0.6</td>
<td>ng</td>
<td>ng</td>
<td>ng</td>
<td>2.1</td>
</tr>
<tr>
<td>Mohanakrishna et al. [100]</td>
<td>Enriched mixed culture</td>
<td>VITO-Core™ with</td>
<td>–0.4</td>
<td>–128</td>
<td>ng</td>
<td>ng</td>
<td>29.91</td>
</tr>
<tr>
<td>Mohanakrishna et al. [101]</td>
<td>Enriched mixed culture</td>
<td>graphite stick</td>
<td>–0.6</td>
<td>–44.89</td>
<td>3.06</td>
<td>ng</td>
<td>30.25</td>
</tr>
<tr>
<td>Jourdin et al. [95]</td>
<td>Enriched mixed culture</td>
<td>MWCN-RVC</td>
<td>–1.1</td>
<td>–200</td>
<td>ng</td>
<td>1330</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>Bajracharya et al. [28]</td>
<td>Enriched mixed culture</td>
<td>Graphite with</td>
<td>–0.9</td>
<td>10</td>
<td>0.12–3.96</td>
<td>10.8–119</td>
<td>35.46–88</td>
</tr>
<tr>
<td>Bajracharya et al. [27]</td>
<td>Enriched mixed culture</td>
<td>Carbon felt with graphite stick</td>
<td>–0.8</td>
<td>10</td>
<td>1.3–6.3</td>
<td>6.67–31.47</td>
<td>16.2–49.4</td>
</tr>
</tbody>
</table>

CE- Coulombic efficiency, RVC-Reticulated vitreous carbon; MWCN- Multi-walled carbon nanotubes; SS- Stainless Steel; WW- Wastewater; ng- Not given or cannot be
deduced; WWTP- Wastewater treatment plant.
a Derived on the basis of total catholyte spent on reported days of operation.
b Calculated based on linear production rate and reported flow rate.
c Calculated including H\(_2\).
sufficient reducing equivalents (H+ or H2) are available [106]. CO2 reduction using mixed culture, at the cathode potential more negative than −0.9 V vs Ag/AgCl, normally results in acetate production but under further reduction, ethanol and butyrate can also be synthesized. When the accumulation of acetate reached more than 1.5 g L−1 in batch operations, the solution pH decreased to below 6 and the reduction products started to shift towards ethanol and butyrate upon decreasing the pH below 6 [27]. Ethanol is produced from acetate reduction at lower pH when the undissociated acids exist [107]. The pH of catholyte can also be lowered by continuous CO2 gas sparging. Supplying 80–100% CO2 mixture with N2 resulted in an increase in butyrate concentration in MES [27]. Along the WL pathway, ethanol and butyrate are also produced either directly from CO2 reduction or by acetate reduction or from both. The thermodynamics of ethanol production by CO2 or acetate reduction indicates that lower pH and excess H2 can shift the production to ethanol. The selectivity of the products of microbial CO2 reduction has been shown to be limited by micro-elements of trace metal and vitamin components due to their effect on the activity of NADPH/NADP and Acetyl-CoA enzymes [108]. By limiting micronutrient and trace elements (namely pantothentic acid and cobalt), higher concentrations of ethanol were produced rather than acetate [109]. This suggests that to enhance the production of longer carboxylates or more reduced compounds, strategies from gas fermentations can be used in MES.

Bioethanol is a readily commercialized renewable fuel contributing towards the reduction of negative environmental impacts generated by fossil fuels. Biocathodic production of ethanol can be possible from acetate and directly from carbon dioxide. Thermodynamically, the reduction of acetate to ethanol is more favorable than the reduction of bicarbonate to ethanol. In a study performed by Steinbusch et al. [110], at an applied cathode potential of –0.35 V (vs SHE) using mixed cultures as biocatalyst with graphite felt electrodes and pH of 5.5, acetate was bioelectrochemically reduced to ethanol. Addition of mediators such as methyl viologen (MV), neutral red (NR), and anthraquinone-2,6-disulfonate (AQDS), showed a positive impact on ethanol synthesis [110]. Earlier studies showed the bioelectrochemical reduction of VFA to alcohols by mixed consortia in presence of H2 under acidic conditions (pH 5) [107]. Solventogenesis metabolism in homoacetogens occurs via the activation of alcohol dehydrogenase [82]. Ethanol and butanol production via the CO2 reduction in MES was reported when acetate/butyrate accumulated in the reactor and went through further reduction. However, microbial utilization of ethanol/butanol in other process kept their concentration quite low in MES [27,70,80].

5.2.2. Glycerol

Production of glycerol from the reduction of CO2 was demonstrated by Soussan et al. [79]. In this study, glycerol production was achieved from CO2 reduction in presence of succinate with G. sulfurreducens as the biocatalyst. Initially fumarate was converted to succinate as in Gregory et al. [111] and then CO2 and succinate were converted to glycerol at an applied potential of −0.4 V vs SHE. Glycerol remained inside the bacterial cells which was released after the polarization potential changed from −0.4 V to −0.2 V vs SHE which created a physiological stress on the bacterial cells [79].

5.3. Gases

5.3.1. Methane

Methane is a product of the energy-generating metabolism in methanogenic archaea which use CO2 as a terminal electron acceptor and hydrogen as an electron source. An enzyme called methyl coenzyme M reductase (MCR) in the archaea catalyzes the final step in the formation of methane [112]. Methanogenesis can be incorporated at the cathode of BES for CO2 reduction with an electrical input, using methanogenic microbes as biocatalysts. The process is called electromethanogenesis [113]. Electrochemically active methanogenic biofilm at the cathode reduces CO2 to methane by using the electrons directly from electrical current. The oxidation reaction at the anode, especially the water oxidation yields protons and electrons. Thus, the production of methane becomes completely autotrophic and electricity-driven, without the use of precious metal catalyst. The half-reactions for bioelectrochemical CO2 reduction to methane in the BES are given below.

At anode. $4\text{H}_2\text{O} \rightarrow 2\text{O}_2 + 8\text{H}^+ + 8\text{e}^- \quad \text{E}^{\circ} = 0.82 \text{ V vs SHE}$

At cathode. $\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad \text{E}^{\circ} = -0.24 \text{ V vs SHE}$

The overall reactions is

$\text{CO}_2 + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + 2\text{O}_2 \quad \text{Cell voltage} = -1.06 \text{ V}$

The potential applied at the cathode to overcome the CO2 reduction overpotential of carbon electrode with aqueous electrolyte can accompany the hydrogen evolution reaction (HER) at the cathode. Electrochemically or bioelectrochemically produced hydrogen at the cathode further assist CO2 reduction by hydrogenotrophic methanogens. Thus, methanogens at the cathode can catalyze the methane producing CO2 reduction either by directly accepting the electrons from the cathode or indirectly via hydrogen as a mediator. Then the reaction becomes

At cathode

$8\text{H}^+ + 8\text{e}^- \rightarrow 4\text{H}_2 \quad \text{E}^{\circ} = -0.411 \text{ V vs SHE}$

$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$

Several studies on methane-producing BES from CO2 reduction at the cathode in two–chamber reactor are overviewed in Table 4. Methane production from CO2 were reported from −0.55 to −1.15 V vs SHE cathode potentials. The performances of BES were measured in terms of methane production rate and the coulombic efficiency (efficiency of electron captured as methane). The reported methane production rates range from 0.12 L m−2 d−1 [114] at −0.55 V vs SHE to 24 L m−2 d−1 [115] at −1.15 V vs SHE. Majority of the studies reported the CO2 reduction to methane between −0.6 and −0.9 V vs SHE which were more negative than the hydrogen evolution potential (−0.41 V vs SHE). Hence the CO2 reduction to methane should be mediated by hydrogen production at the cathode. Nevertheless, methane production with direct transfer of electrons from cathode to the microbes was also explained in a number of studies which reported significantly higher current densities at ≤ −0.8 V vs SHE than the abiotic cathode producing hydrogen. The higher current densities were justified as a result of direct electron transfer to the microbes in CO2 reduction [113,116]. The CE of methane production were normally more than 70% when the reduction was achieved at less negative cathode potentials; almost 95–99% CE were reported at −0.7 to −0.8 V vs SHE [113,117]. A few exceptions were also reported with low efficiency of methane production for example only 23% CE at −0.55 V vs SHE [114] and 55% CE at −0.59 V vs SHE [69].

Methane production rates were considerably high at more negative potential (hydrogen evolving potentials) when molecular hydrogen mediates the methane production from CO2. Higher
energy inputs resulted into higher production rates but the coulombic efficiencies of the process were relatively low 57% [115] and 80% [116] (Fig. 5). A large fraction of electrons goes into proton reduction and considerable amount of hydrogen gas evolved, escaped the reactor without being utilized.

Fig. 5 shows a significant increase in the methane production rates and current densities at more negative potentials than −0.7 V whereas the coulombic efficiencies are higher at −0.7 to −0.9 V vs SHE. The box marked in Fig. 5 highlights the studies reporting CO₂ reduction to methane at high rates and with high efficiencies. Direct electron transfer from cathode to the methanogens was most likely at −0.7 to −0.85 V vs SHE due to the high current. Indirect electron transfer might also be possible with the small amount of hydrogen mediating the CO₂ reduction. The use of electrical energy from renewable sources by methanogenic biocathode for methane production would make the system truly sustainable. In another sense, this system enables the storage of excess electricity from intermittent sources like solar or wind as methane that could be later reused to generate electricity or used as a fuel.

Under the direct electron transfer CO₂ reduction to methane with water oxidation as electron source, the minimum thermodynamic energy input required is 32.7 MJ per m³ CH₄ (11 kWh m⁻³ CH₄) while operating at standard biological condition at pH 7 and 25°C [118]. When methane is produced by biological CO₂ reduction with the external supply of hydrogen, the required power input will be 25.5 kWh m⁻³ CH₄ [119]. Hence, direct extracellular electron transfer would be the most energy-efficient process for CO₂ reduction to methane. However, most of the literature reports CO₂ reduction to methane with direct as well as indirect electron transfer through small amount of hydrogen production at slightly more negative cathode potentials than −0.7 V vs SHE. Accompanying both direct and indirect electron transfer in CO₂ reduction to methane, van Eerten-Jansen et al. [114] reported a power input of 18.2 kWh m⁻³ CH₄ at −0.7 V cathode potential which was still lower than the power input with the external hydrogen supply.

5.3.2. Ethylene

Ethylene is the most extensively used organic compound, produced exclusively from fossil fuels in chemical industries which emits large amount of GHGs. Biological approaches have been prospected as sustainable process to produce ethylene. Ethylene is naturally synthesized in plants as a plant hormone which is especially known for regulating fruit ripening and other biological processes.

Production of ethylene from CO₂ is a highly attractive approach as the process is conceptually carbon neutral. Electrochemical reduction of CO₂ to ethylene has been reported in aqueous electrolyte using Cu electrodes [123,124]. But due to the lower energy

Table 4
Overview of the performances of methane producing biocathodes from CO₂ in two-chamber bioelectrochemical reactor.

<table>
<thead>
<tr>
<th>Cathode material</th>
<th>Inoculum source</th>
<th>E₉₋₁V vs SHE</th>
<th>Current density A m⁻²</th>
<th>Methane production rate (L m⁻³ d⁻¹)</th>
<th>CE %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon cloth</td>
<td>Enriched culture activated sludge WWTP</td>
<td>–0.8</td>
<td>1.8</td>
<td>4.5</td>
<td>96</td>
<td>[113]</td>
</tr>
<tr>
<td>Carbon paper</td>
<td>Enriched culture anaerobic sludge packed bed biofilm reactor fed fatty acids</td>
<td>–0.9</td>
<td>2.9</td>
<td>9.2</td>
<td>80</td>
<td>[116]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Non-enriched culture (anaerobic sludge UASB treating distillery WW)</td>
<td>–0.7</td>
<td>1.75</td>
<td>4.7</td>
<td>95.2</td>
<td>[114]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Enriched culture (brewery wastewater)</td>
<td>–0.59</td>
<td>n.r.</td>
<td>7 mmol L⁻¹ d⁻¹</td>
<td>55</td>
<td>[69]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Non-enriched culture (anaerobic sludge UASB treating distillery WW)</td>
<td>–0.55</td>
<td>0.2</td>
<td>0.12</td>
<td>23</td>
<td>[114]</td>
</tr>
<tr>
<td>Carbon felt</td>
<td>Enriched culture activated sludge WWTP</td>
<td>–0.75</td>
<td>3.4</td>
<td>6.7</td>
<td>89</td>
<td>[115]</td>
</tr>
<tr>
<td>Carbon felt</td>
<td>Enriched culture anaerobic sludge WWTP</td>
<td>–1.15</td>
<td>15</td>
<td>24</td>
<td>57</td>
<td>[115]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Non-enriched culture anaerobic sludge WWTP</td>
<td>–0.7</td>
<td>2.9</td>
<td>5.2</td>
<td>73</td>
<td>[118]</td>
</tr>
<tr>
<td>Carbon stick</td>
<td>Archaeon strain IM1</td>
<td>–0.7</td>
<td>NR</td>
<td>10.03 ± 1.4</td>
<td>24.2 ± 4.7</td>
<td>[120]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Anaerobic sludge suspension</td>
<td>–0.94</td>
<td>0.037 A⁻¹</td>
<td>4.2 ± 0.3 mmol L⁻¹ d⁻¹</td>
<td>84 ± 3</td>
<td>[122]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Anaerobic sludge</td>
<td>–1.22</td>
<td>0.091 A⁻¹</td>
<td>9.7 ± 0.6 mmol L⁻¹ d⁻¹</td>
<td>90 ± 5</td>
<td>[122]</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>Anaerobic sludge</td>
<td>–1.41d</td>
<td>–0.12 A⁻¹</td>
<td>12 mmol L⁻¹ d⁻¹</td>
<td>72–90</td>
<td>[122]</td>
</tr>
</tbody>
</table>

NG: not reported.

a Unnormalized current density.
b Anode set at –0.2 V vs SHE.
c Anode set at −0.1 V vs SHE.
d Anode set at 0.2 V vs SHE.

Fig. 5. Methane production rate (in log scale), current density (in log scale) and coulombic efficiency (CE) of bioelectrochemical CO₂ reduction with respect to applied cathode potential. The figure is prepared on the basis of methane productions reported in various literature reports.
efficiency and lower selectivity of electrochemical approach, biological catalysts are required to make the process more sustainable.

A few chemolithotrophic *Thiobacillus ferroxidans* strains were reported to produce ethylene but the ethylene-forming enzyme (EFE) system have not yet been characterized [125]. Several microbes including some species of chemolithotrophs are capable of producing ethylene from methionine in a two-step reaction via KMBA (2-keto-4-methylthiobutyric acid) pathway [125] whereas *Pseudomonas syringae* group directly synthesized a larger amount of ethylene from α-ketoglutarate and arginine as substrates in a one-step reaction catalyzed by the EFE [125,126]. In the photosynthetic approach, CO₂ is fixed in the cyanobacterial cell via the calvin cycle into acetyl-CoA which is further used in tricarboxylic acid cycle for the production ethylene from α-ketoglutarate (Fig. 6). Continuous ethylene production from CO₂ in photosynthetic process using genetically engineered cyanobacterial strain *Synechocystis* sp. PCC 6803 has reported peak ethylene production rate of 171 mg L⁻¹ day⁻¹ [127].

Direct ethylene production from CO₂ in bioelectrochemical reduction has not been reported till date. However, ethylene production via bioelectrochemical CO₂ can be achieved by using chemolithotrophic bacteria like *Thiobacillus ferroxidans* that possesses ethylene production system or by supplementing methionine in the chemolithotrophic growth media via KMBA pathway. A two-stage bioelectrochemical ethylene production from CO₂ can be carried out by combining (1) acetyl CoA (acetate) generation via bioelectrochemical CO₂ reduction and (2) production of ethylene from the acetyl CoA via the TCA cycle.

**6. Current challenges for CO₂ reduction in MES technology**

**6.1. Robust and stable biocathode**

Biocathode having robust EABs with CO₂ reducing capability is a prerequisite for efficient CO₂ reduction in MES. Acetogens from mixed culture inoculum have been used in MES that can primarily produces acetate from CO₂ reduction by consuming the reducing equivalents available from the electric input or the electrochemically produced hydrogen [64,88,98]. However, the development of electroactive CO₂ reducing biofilms which are stable over longer periods are the major challenges in MES technology. CO₂ reduction using acetogenic biofilm will also produce methane, if not suppressed and this results in lower product selectivity and yields [69] [115]. Methane production in the microbial catalysis of CO₂ reduction should also be prevented in order to shift the bioprocess towards the production of other multi-carbon compounds. The evolution of methane or hydrogen from the biocathode can also destabilize the biofilm resulting in decreased product titers. High product titers are also necessary to lower the downstream processing costs.

Pre-treatment of the mixed culture inocula were carried out to develop the required biocathode in MES. Enrichment of acetogens by short-term inhibition of methanogens using sodium-2-bromoethanesulfonate (Na-BES) in MES reactor was reported in a number of literature [64,94,97,128]. But the continual input of such inhibitors is not sustainable for long-term and large-scale applications [29]. A strategy of methanogen elimination with the one-time addition of Na-BES and successive enrichment was also reported recently [88] which showed that the enriched culture had 77% of the relative abundance of *Clostridium* species and only < 0.1% relative abundance of methanogens. But during the MES operation, methanogenic activity was avoided only for two to three months [88]. In another enrichment method, methanogenesis inhibitor was used only once, after applying heat-shock and a successive culture under H₂:CO₂ created a suitable inoculum for CO₂ reduction and avoided methanogen for long-term MES operation [27]. A successful suppression of methanorganic activity and the accumulation of acetate to higher levels confirmed an effective biocathode towards CO₂ reduction which is a promising outcome for the advancement of MES.

The microorganisms thriving on DET from the electrode or on hydrogen transfer or those utilizing acetate as the substrate can all co-exists in the same MES biofilm when adapted in the dynamic cathode conditions. Such microbiome results in a stable and self-maintaining biocathode capable of reducing CO₂ over longer terms [27]. Hence, the mixture of strains can be effectively shaped as a biocatalyst for CO₂ reduction in long-term operating MES.

**6.2. Faster adaptation to electron uptake**

The yields in microbial bioproduction are governed by the metabolic activities of the organisms and the metabolism is potentially limited by (1) energy source, (2) redox power and (3) carbon source [129]. In microbial metabolism, energy is conserved as adenosine triphosphate (ATP) and the redox power as nicotinamide adenine dinucleotide (phosphate) [NAD(P)H]. Wood-Ljungdahl (WL) pathway is the most energetically efficient pathway known for CO₂ reduction as more than 95% of carbon and electron flow is diverted to the production of extracellular end-products, rather than to the microbial growth and biomass production [130]. There is no net energy gained during the CO₂ reduction based on the WL pathway so the bacterial metabolism could be limited. Henceforth, microbes having other CO₂ reduction pathways also would be helpful to balance the net energy gain for overall microbiome. Selective enrichment of one source of mixed culture in different conditions that allows only strains having designated characteristic for MES, namely facultative, autotrophic, electro-active, biofilm forming, etc., and mixing them into one culture at the end will be a good strategy to have a faster adaptation for effective CO₂ reduction. Furthermore, in the autotrophic growth of acetogens utilizing the mineral medium, the cell biomass yield
and growth are normally reduced due to the nutritional limitation. Nutritional supplements such as yeast extract were shown to enhance the cell growth of acetogens in H₂:CO₂ culture [131]. For a fast growth and adaptation, nutrient supplements can be useful but it is worthy to investigate whether the exclusion of nutrients supplements can be achieved to make the MES economically more attractive.

6.3. Understanding ET mechanism

Several acetogenic species, including *S. ovata* and *C. ljungdahlii*, catalyze the CO₂ reduction at −0.4 V vs SHE ahead of H₂ evolution [24,71]. However, the mechanism of bacterial interaction with the solid electrode is still need to be investigated for CO₂ reduction. Under the actual reaction conditions, the overpotential of the electrode imposes barriers demanding much lower cathode potential. The overpotential of −0.28 V was reported for the H₂ producing graphite felt biocathode at neutral pH [132]. The overpotential for CO₂ reduction cathode might require lower cathode potential beyond hydrogen evolution. Moreover, it is equally likely that an undefined mixed culture biocatalyst first catalyzes H₂ evolution and then H₂ mediates CO₂ reduction. Reduction via DET is interesting if higher production rates can be achieved. However, electricity-driven production needs optimization with minimum electric power input regardless of the mechanisms of DET or H₂ mediated transfer.

Research on the activation of enzymes and proteins like cytochrome-c for extracellular electron transfer on homoacetogenic bacteria show the possibility of multiple mechanisms of electron transfer depending on each bacterial species. Sydow et al. [133] found that, in *S. ovata* cytochrome-c was present in the outer membrane of the bacteria which favors the DET whereas in another homoacetogen, *C. ljungdahlii*, no cytochrome-c was available in the cell-membrane. However, hydrogenases are reported to carry out the reduction of CO₂ in *C. ljungdahlii*, so the electron transfer should be via H₂ in *C. ljungdahlii* biocathode [133].

In most of the literature, the bioelectrochemical reduction of CO₂ was reported to occur at much lower cathode potentials than the theoretical H₂ evolution potential (−0.414 V vs SHE or −0.61 V vs Ag/AgCl). MES experiments showed a considerable production of acetate only when the polarization potentials were more negative than −0.8 V vs Ag/AgCl and whenever the potentials were above or equal to −0.8 V vs Ag/AgCl, acetate and butyrate were degraded instead of CO₂ reduction [27]. As the acetate production occurs only below −0.9 V vs Ag/AgCl, H₂ should be mediating the CO₂ reduction reaction [27]. Higher current densities (up to 10 A m⁻²) obtained at −0.9 to −1 V vs Ag/AgCl with biocathode is an indication of microbial electrocatalysis in combination with H₂ evolution. But the actual mechanism of electron transfer in undefined mixed culture biocathode is difficult to investigate in detail. Fig. 7 illustrates the possible mechanism of bacterial interaction with the cathode which was proposed previously by Blanchet et al. [70]. Direct electron transfer mechanism can also occur beyond the hydrogen evolution onset potentials. Indeed, DET and simultaneous H₂ formation at cathode governs typical electron transfer to the biocatalysts. Based on the works by Bajracharya et al. [27] and Jourdin et al. [94], the H₂ interaction in MES resembles the case depicted in the middle diagram of Fig. 7. The on-site produced H₂ would be effective for CO₂ reduction as it is immediately available to the bacteria. However, the high rate of electrochemical H₂ evolution at cathode might disrupt the biofilm formation. Overall, the electrochemical or biocatalyzed H₂ evolution can support high rate bioproduction from CO₂ reduction but the CEs might remain low.

6.4. Reactor designs to maximize mass-transfer and biofilm formation

The improvement in CE of CO₂ based MES systems can be achieved by maximizing the gaseous substrates and energy source (H₂) availability along with the bacterial adaptation. The bioelectrochemical reactors could be adapted into the fermentative bioreactors configurations for the optimization of biological retention and mass-transfer of substrates. Roy et al. [134] proposed possible modifications in the reactor shapes, electrode configurations and liquid/gas interfaces for MES to maximize gas utilization. Immobilization of cells to favor biofilm formation on the electrode is one of the most applied approaches. Electrically conductive materials, like activated carbon, and carbon nanotubes can be used in the electrodes to ensure electron transport to/from the active biofilm over the large electrode surface with minimum resistance. Notably, the high volumetric production rate of acetate from CO₂ reduction in MES, reported by Marshall et al. [64], was indeed achieved by using graphite granules bed as a cathode. Packed or fluidized bed configurations and biofilters are the possible modifications in bioelectrochemical reactors which can favor biofilm formation and simultaneously permit the gas or liquid substrates to flow through the immobilized cells [134]. Incorporating the gas fermentation reactors design in the CO₂ reducing MES will allow high microbial cell retention and improves gas-liquid mass-transfer to enhance the CO₂ reduction rates and efficiencies.

![Fig. 7. Possible mechanisms of electron transfer for CO₂ reduction at cathode (Adapted from Blanchet et al. [70])](Image)
7. Future outlook of CO2 conversion in MES

7.1. Diversification of end products for economic viability

A wide range of fuels as well as commodity chemicals can be potentially synthesized in series of MES processes starting with CO2 reduction. Acetate and methane are the most widely produced and very common precursors for chemical synthesis that can be initially derived from CO2 reduction based on the type of biocatalysts. High value and special products are sought in MES system to achieve economic advantages from the production system. The implementation of MES technology will be attractive only when the end product can be diversified with high titers and purity.

Acetyl-CoA is the crucial intermediate in the WL pathway of CO2 reduction which is also a main precursor for the production of various commodity chemicals [78]. Along the WL pathway, several strains of acetogens are reported to produce high titers of ethanol, butyrate and in some instances lactate, 1,3-propanediol and butanol under the appropriate conditions [78,135]. Production of ethanol, propionate and butyrate from CO2 was also achieved in the MES [27,28]. Chain-elongation mechanism in microorganism produces propionate (C3), butyrate (C4) and caproate (C6) from acetate under H2:CO2 fermentation [102,103]. Hence, chain elongation can also be incorporated in MES for the diversification of end product. Further reduction of carboxylic acids to alcohols also occurs when solventogenesis stress are imposed by shifting the operational conditions. In addition, the end-product can go to lactate (C3) and succinate (C4) under Krebs cycle. Fig. 8 portrays an overview of possible end-products formed in MES from CO2 reduction via the WL pathways. These value-added products have direct applications or can be further utilized as substrates or precursors for other chemical/fuel production processes. Selective enrichment of the biocatalyst, identifying the suitable strains and optimization of the operating conditions for the specific product formation will enhance the chances of producing commercially viable chemicals with higher carbon number. Determined outlook is required from researchers of different disciplines towards upscaling of the technology, as it is one of the most overwhelmingly trans-disciplinary systems ever studied.

7.2. Integration of product separation and purification

An important process constraint in MES is feedback/product inhibition or microbial tolerance towards the specific product accumulation. Beyond certain concentration of any specific product, the microbial metabolism switches over to avoid the toxic effect of that product. The feedback inhibition will impede the continuous trend of production, limiting the titer to ppm level or at lower percentages. Low product titer in MES process brings down the economic viability of the process. Integrated product separation/extraction system that allows effective product recovery even at low titer without hindering the MES process is a promising strategy to reduce product inhibition and additionally to achieve product recovery in MES process.

Membrane electrosynthesis has been reported to extract short/mid-chain carboxylates (e.g. acetate, butyrate, caproate, etc.) by transferring them across an anion exchange membrane (AEM) [137]. An integrated approach of application of membrane electrosynthesis within the CO2 reducing MES reactor has succeeded to separate and concentrate acetic acid to high level at the same time [89].
Gildemyn et al. [89] reported an acetate production rate of 11.6 mM d$^{-1}$ from CO$_2$ reduction by integrating the in situ acetate separation in MES with membrane electrolysis and the acetic acid was transported through AEM and had accumulated up to 13.5 g L$^{-1}$ in a separate compartment after transported through AEM. However, the requirement of high electric energy input for the extraction and the fouling of membranes are some of the shortcomings of this approach. Another approach of in situ acetate separation in MES was demonstrated based on the application of sorption of acetate in anion-exchange resins [138,139]. Acetate sorption of 10–18 mg g-resin$^{-1}$ was observed from the MES catholyte. In case of anion-exchange resin sorption, extra energy input was not required but the resin regenerants, such as hydroxide or carbonate solutions are required to recover the adsorbed carboxylate anions. The requirement of large quantities of concentrated regenerant solutions might pose economical constraints in the large-scale application of sorption strategy. In addition, the fouling and clogging in the resin column can constrain the whole MES process.

Further development of MES reactor with integrated separation system is indispensable for the economic feasibility of application of MES process and recovery of the products from CO$_2$ reduction. A full production and separation system should be evaluated at an industrial scale and the product must be tested for it suitability for the eventual application.

7.3. Prospects of MES in biorefineries

MES technology offers numerous potential prospects with smart innovations by converting electrical energy to new value-added chemicals or biofuels. MES is a promising technology that can store electricity directly as biobased energy carriers or their precursor chemicals. Unlike other biomass to biofuel approaches, this electricity-driven bioproduction method is independent of fertile land. Photovoltaic system converts solar energy to electricity with an efficiency of around 10–15%, while the net photosynthetic efficiency of plants in organic matter production is around 0.5%. When an MES system is operated with an electric input from photovoltaic system, then even assuming 25% efficiency of MES, the overall organic matter production efficiency will be 3.75%. This means, at least 7.5 times more organic matter can be harvested per m$^2$ of land when the bioproduction is carried out in MES powered by photovoltaic system. Thus, the land-area requirement for the bioproduction in MES becomes appreciably low and need not to be fertile. Large volumes of fresh water and nutrients input can be avoided in the bioproduction of chemicals as compared to agricultural production. These are the remarkable advantages of microbial electrosynthesis, apart from being a clean technology.

Microbial electrosynthesis could serve as a sustainable alternative to the conventional chemical synthesis processes which requires high input of hazardous and non-renewable raw materials in unsustainable manner (high temperature/pressure, acid/alkaline solutions, etc.). Inexpensive and abundant carbon sources including CO$_2$ and waste streams can be utilized for bioproduction with the input of electrical energy only. In addition, MES offers unique possibilities to promote cell growth and the controlling of redox state in bioprocesses by steering the metabolic pathways electrically. A concept for the integration of MES systems within the biobased conversions in industrial scale is depicted in Fig. 9. MES puts forward a closed-loop model in biorefineries concept offering a wide range of platform chemicals by recycling and reutilizing the waste materials and gases from effluents and emissions. Therefore, this is a promising technology that provides prospects of renewable energy-driven waste to fuel/chemical to establish a circular biobased economy. The proof-of-principle of integration of the bioelectrochemical systems into the biorefineries has been employed and are proved to be technologically feasible [140]. For the scaled-up applications, further understanding and technological advancements are still underway.

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