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Graphical Abstract

Current Trends in Enzymatic Electrosynthesis for CO₂ Reduction

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Abstract

Enzymatic electrosynthesis offers a novel approach to the production of chemicals through CO₂ sequestration. In this mini-review, we present the most recent state-of-the-art information on enzymatic CO₂ reduction for the production of chemicals such as formic acid using oxidoreductase (single or multiple) enzymes as electrocatalysts in enzymatic electrosynthesis cell (EEC). Key challenges towards upscaling of this CO₂ utilization approach are identified and future research directions are discussed briefly.

Key words: Enzymatic electrosynthesis, formic acid, oxidoreductase, CO₂ reduction

1. Introduction

Climate change due to the precipitous increase in greenhouse gas emissions particularly CO₂ in the atmosphere is driving the interest in developing carbon capture and utilization (CCU) technologies. CO₂ is the major carbon source which can be transformed into useful chemicals and fuels using different CCU technologies. Biological conversion of CO₂ is considered as a viable and sustainable approach for transitioning the existing linear carbon economy into a circular one in a highly desired biorefinery framework. The major biological CO₂ conversion approaches include microalgae cultivation, gas fermentation and microbial electrosynthesis [1-5]. The former is based on the use of algae and photosynthetic bacteria, whereas the latter two use chemolithoautotrophic microbial catalysts. Microbial electrosynthesis is an electricity-driven CO₂ reduction process catalyzed by microorganisms at the cathode of the bioelectrochemical systems [6]. Although the use of the whole cell microbial catalysts offers some important advantages, it also brings along several metabolism and process-related challenges. For instance, it results in less productivity due to the mass transport losses during the translocation of redox mediators across the cellular membrane and activation losses during the long electron transfer pathways [7].

Enzymatic electrosynthesis is another bioelectrochemical CO₂ reduction approach which is based on the use of specific enzyme catalysts instead of whole cell biocatalysts (Fig. 1).

Figure 1: Schematic representation of a typical enzymatic electrosynthesis cell.

The renewable energy systems can supply the electrical energy needed to facilitate the reduction reactions at the cathodes. The use of specific enzymes to catalyze the cathodic CO_2 reduction to produce specific products overcomes different losses. It is mainly due to the absence of interferences and intrinsic resistances along with the cost-effective downstream process [8-10]. Enzymatic electrosynthesis can thus be a remarkably energy efficient process. In this process, single or multiple enzyme (cascade-type) catalysts can be used depending upon the target product [11].

A major fraction of enzymes involved in CO_2 reduction discovered to date belongs to the oxidoreductase family and carbonic anhydrase (CA) [12,13]. These enzymes are vital for a vast choice of reactions in biological systems, primarily redox reactions. Usually, these enzymes exhibit a ping-pong mechanism where addition or loss of electrons and few atoms (involved in oxidation or reduction) are dispensing the unsteady enzyme form. Further, the enzyme gets to its unchanged form upon completing the reaction. This reaction is often accelerated by cofactors such as PQQ, FAD, heme and transition metals that bind firmly to the enzyme or a co-substrate such as NADP^+ , NAD^+ , and FMN that bind temporarily for short-duration to accomplish the reaction. These cofactors or chemical species loose or gain temporarily stored electrons to proceed with the redox reaction [14-16]. In recent times, the most studied single enzyme process has been the synthesis of formic acid from CO_2 reduction [10] using formate dehydrogenases (FDH) along with co-factor NADH [17, 18]. CA has been

used for less energy-intensive desorption of CO₂ scrubber for solvent recovery [19, 20]. The multi-enzyme approach is more advantageous over the single enzyme systems. It facilitates high CO₂ solubility (HCO₃³⁻) by CA in the aqueous phase. Later HCO₃³⁻ is converted to formic acid by FDH [21].

2. Recent advancements in Enzymatic electrosynthesis from CO₂

A classic enzymatic electrosynthesis cell (EEC) consists of enzymatic bioanode and biocathode (electrocatalysts) separated by electrolyte or gel or polymer and an external circuit with the load or power supply. Solid electrodes of different materials act as conductors and support the enzymes immobilization. The electrocatalysts used in EEC are oxidoreductase enzymes - a class of enzymes that catalyze redox reactions. Since these enzymes are selective electrocatalysts, careful selection of the specific enzyme is critical for the conversion of CO₂ to chemicals. Formic acid production has been the main target process thus far due to its commercial importance [22]. Other target products include methane or methanol [23]. Very limited research work has been reported since the last two years on enzymatic electrosynthesis of formic acid from CO₂ reduction at cathode using FDH as a catalyst and NADH as an electron shuttle (Table 1).

Table 1. A consolidated table on the formic acid production in the enzymatic electrochemical cell through CO₂ reduction

S. No	System	Enzyme	Enzyme Source	Cathodic reaction	Product	Maximum Concentration/ production rate	Ref.
1	EEC	FDH+CA	<i>Candida bodoinii</i> (FDH)+ bovine erythrocytes (CA)	CA increases the solubility of CO ₂ + FDH reduces CO ₂ to formic acid with NADH e- shuttle	Formic acid	86.26 mg L ⁻¹ h ⁻¹	[10]
2	Hybrid MFC-EFC system	Cb- FDH	<i>Candida bodoinii</i>	FDH reduces CO ₂ to formic acid with NADH e- shuttle	Formate	60 mg L ⁻¹ h ⁻¹	[24]
3	EEC	Hdr-SC	<i>Methanococcus maripaludis</i>	FDH reduces CO ₂ to formic acid with Hdr-SC e- shuttle	Formate	12 mg L ⁻¹ h ⁻¹	[25]
4	EEC	FDH	<i>Candida bodoinii</i>	FDH reduces CO ₂ to formic acid with NADH e- shuttle	Formic acid	225.81 mg L ⁻¹ h ⁻¹	[26]
5	Electro-enzymatic system	RcFDH	<i>Rhodobacter capsulatus</i>	RcFDH reduces CO ₂ to formic acid with methyl viologen (MV) e- shuttle	Formate	276 mg L ⁻¹	[27]
6	Electro-enzymatic system	molybdenum-FDH (Mo-FDH)	<i>Escherichia coli</i>	Mo-FDH reduces CO ₂ to formate with Cobaltocene e ⁻ shuttle	Formate		[34]

7	Microbial electrosynthesis system (MES)	FDH	<i>Ralstonia eutropha</i>	neutral red (NR) and NADH	poly(3-hydroxybutyrate) (PHB)	$485 \pm 13 \text{ mg L}^{-1}$	[38]
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A recent study by Zhang and coworkers investigated enzymatic electrosynthesis of formate from the CO₂ reduction in a hybrid microbial fuel cell-enzymatic fuel cell (MFC-EFC) system. Electrons were harnessed by the degradation of organic pollutants present in the wastewater by anodic electrochemical active bacteria in MFC. In the same system, immobilized cathode surface with FDH extracted from *Candida boidinii* was used along with electro-polymerization and NADH to increase the electrochemical property. The production of formate from NaHCO₃ as the CO₂ source was studied at lowered overpotentials as low as 0.1 V by connecting the MFC stack in different connection modes (i.e., series and parallel) for external power supply. These authors achieved a maximum formate production rate of 60 mg L⁻¹ h⁻¹ with 70% Faradaic efficiency [24]. For the first time, Lienemann and colleagues identified and studied the multi-enzyme heterodisulfide reductase supercomplex (Hdr-SC) in *Methanococcus maripaludis* for rapid formate production. They reported that Hdr-SC catalyze the methanogenesis via direct electron uptake with fast H₂ and formate production in electrochemical reactors at -800 mV vs. Ag/AgCl applied potential. Formate production with 90% coulombic efficiency after five days was achieved [25].

A recent study by Srikanth et al. reported optimized potential (-0.8 V) for CO₂ reduction to formic acid with 12.74% current efficiency at a production rate of 225.81 mg L⁻¹ h⁻¹. In this study, an enzymatic electrosynthesis cell consisting of a H-type two-chambered reactor with anode and cathode was used. The graphite-based cathode was immobilized with FDH as a biocatalyst to convert CO₂ to formic acid. The major limitation of the study was denaturation of the enzyme which limited the production time for 40 min due to non-recycling of the proton donor NADH [26]. With this basic understanding, the same group continued testing the formic acid production using FDH alone and in combination with CA in a dual chambered enzymatic electrochemical cell. It consisted of CO₂ reducing cathode [cold rolled graphite-polytetrafluoroethylene (PTFE) composite layer on a stainless-steel mesh, VITO[®] CORE type electrode] and a platinum wire as the anode. CA was used to increase the solubility of CO₂ and to enhance the formic acid production with both free and immobilized forms (FDH, FDH+CA) on a cold rolled electrode based on graphite powder. Higher production rate (43.13 mg L_{CO₂}⁻¹) and titer (647 mg L⁻¹) were obtained with FDH+CA free-form than other variations studied. Authors identified large variation in the reduction current operation with free form [(-6.2:3.9) Am⁻²], whereas the immobilized form showed less variation [(-3.8:0.5) Am⁻²] due to increased enzyme stability. Moreover, the reproducibility of the data reflected the longevity of the enzyme after immobilization. The addition of CA with FDH increased the consumption of the current in both forms because it allowed rapid dissolution of CO₂, which made it available for the catalytic reaction to increase formic acid production [10]. Choi and co-workers selected O₂ stable FDH, isolated from *Rhodobacter capsulatus* RcfDH) and studied with a different mediator (44 μM of alizarin red S (ARS), anthraquinone-2-sulfonic acid (AQ2S), benzyl viologen (BV), and methyl viologen (MV)) for effective electron transfer to reduce CO₂. An electro-enzymatic system with RcfDH with MV showed an effective CO₂ reduction of 6 mM of formate in 5 hours [27]. A novel CA coated pectin membrane was developed, and its behavior studied for the first time by Nemestóthy and colleagues in the field of membrane technology to separate CO₂/N₂. Authors

prepared a supported liquid membrane (SLM) and identified [Bmim] [NTf₂] ionic liquid (IL) filled with cellulose acetate in the pores for solvent support during SLM fabrication on the enzyme which in turn, caused a quick loss of initial biocatalyst activity. A 3-fold enhancement in higher transmembrane pressures compared to control was observed after improved resistance against high pressure of nearly 7.2 bar. Performance of the CA coated pectin membranes tested with single and mixed gases of CO₂ and N₂ showed markedly increased CO₂ permeability by 93 Barrer, whereas, N₂ remained unaffected [28]. Jin et al. worked on metalorganic frameworks (MOFs) (ZIF-100 and CFA-1) mimicking CA (Zn as an active center in metalloprotein) for effective CO₂ conversion. Authors revealed that MOFs were more efficient for in situ CO₂ conversion than porous materials viz., MCM-41 and activated carbon [29].

In addition to the above-mentioned enzymatic CO₂ conversion, Wang and co-researchers have explored carbon monoxide dehydrogenase (CODH) for CO₂ conversion. In this study researcher screened two different CODH using protein-film electrochemistry and evaluated them in the presence of CO₂ [30]. Hansen et al. assessed the enzymatic electrochemical approach based on the reduction of CO₂ to CO due to central metals in CODH [31]. Amao and Shuto defined the electron shuttling with methyl viologen in enzymatic electrochemical reactions and studied the artificial photosynthesis approach for CO₂ reduction. In this study FDH was coupled to methyl viologen with a long alkyl chain, in turn allied to an indium tin oxide (ITO) electrode. This approach for an artificial photosynthesis route, also includes formate production from CO₂ [32]. Minteer and co-workers recently studied the multienzyme approach to transfer electrons in a cascade way to generate biofuel. In this study, methanol was produced by coupling ADH to NADH regeneration [33]. Bassegoda et al. worked on the heterogeneous enzymatic electrochemical reduction of CO₂ to formate avoiding a co-factor. In the same study, they also focused on active metal site of molybdenum-FDH and revealed highly electrochemically active site than tungsten- FDH in the conversion of CO₂ to formate [34]. Yuan et al. took the lead from previous understanding on active metal site of molybdenum-FDH (Mo-FDH) and extended his work to using Mo-FDH from *Escherichia coli* on the surface of a carbon electrode. In this case, cobaltocene (grafted to poly-(allylamine), Cc-PAA), a low-potential redox polymer was used as an electron mediator for efficient CO₂ reduction. During this study authors achieved 99.5% Faradaic efficiency at 0.66 V (vs. SHE) applied potential [35]. Understanding of heterogeneous enzymatic electrochemical catalytic reduction of CO₂ led to another product formation other than formate. Schlager and coworkers immobilized carbon felt electrode with all the three DH encapsulated alginate matrix avoiding co-factors and studied the reduction of CO₂ to methanol with 40% Faradaic yield [36, 37]. Chen and co-researchers also worked on poly(3-hydroxybutyrate) (PHB) production with genetically engineered ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in *Ralstonia eutropha* to enhance CO₂ fixation. Reactor systems were operated at a constant potential of -0.6 V vs. Ag/AgCl with FDH, neutral red (NR) and NADH along with genetically modified *R. eutropha* at the cathode. This approach showed high PHB production than control system (wild-type *R. eutropha* absence of FDH and NR) [38].

3. Key challenges and future research directions

Despite the low yields, the most reported chemical compound in enzymatic electrosynthesis cell is formic acid production by CO₂ reduction. Most importantly, the research work in this area has been rather limited so far. Several challenges are foreseen towards practical

applicability of enzymatic electrosynthesis cell for formic acid production or other chemical products such as CO, methane, and bicarbonate from CO₂ reduction. Identifying the suitable and efficient microorganisms to produce enzymes and catalyze the reduction reactions efficiently are the prime challenges that need to be addressed. Apart from that fundamental challenges like high ohmic and activation losses, concentration over-potentials at the cathode need to be reduced for efficient CO₂ conversion. Another important challenge in engineering aspects is to design an economically viable enzymatic electrochemical system for CO₂ conversion into liquid fuels and chemicals. Other challenges include cofactor enzyme generation system and efficient hydration of CO₂ in water. The long-term stability of enzyme catalysts also needs to be considered to make the system work for longer times. Towards the enhancement of the catalytic reaction, efficient approach for the preparation of enzymes, optimization of the reaction and stability mechanisms needs to be considered. The sensitivity of the enzymes in harsh and high intense sunlight conditions, which decrease the enzymatic activity is another area that needs research focus. The cost related aspects include protein purification which hinders the large-scale application of such enzymatic systems also needs to be addressed.

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References

- [1] S.A. Patil, S. Gildemyn, D. Pant, K. Zengler, B.E Logan, K. Rabaey, A logical data representation framework for electricity-driven bioproduction processes. *Biotechnol. Adv.* (2015) 33, 736-744.
- [2] P. Chiranjeevi, S. Venkata Mohan., Critical parametric influence on microalgae cultivation towards maximizing biomass growth with simultaneous lipid productivity. *Renew Energy.*, (2016) 98, 64–71.
- [3] F.M Liew, M.E Martin, R.C Tappel, B.D Heijstra, C. Mihalcea, and M. Kopke., Gas Fermentation-A Flexible Platform for Commercial Scale Production of Low-Carbon-Fuels and Chemicals from Waste and Renewable Feedstocks. *Frontiers in Microbiology.*, (2016) 7, 694.
- [4] S. Bajracharya, K. Vanbroekhoven, C.J.N Buisman, D.P.B.T.B Strik, D. Pant, Bioelectrochemical conversion of CO₂ to chemicals: CO₂ as a next-generation feedstock for electricity-driven bioproduction in batch and continuous modes. *Faraday Discuss.*, (2017) 202, 433-449.
- [5] O. G. Sánchez, Y.Y. Birdja, M. Bulut, J. Vaes, T. Breugelmans, D. Pant, Recent advances in industrial CO₂ electroreduction, *Current Opinion in Green and Sustainable Chemistry*, (2019) <https://doi.org/10.1016/j.cogsc.2019.01.005>.
- * This paper gives the most recent updates of electrocatalytic CO₂ reduction, its upscaling and the efforts at industrial scale.
- [6] S. Bajracharya, S. Srikanth, G. Mohanakrishna, R. Zacharia, D.P. Strik, and D. Pant., Biotransformation of carbon dioxide in bioelectrochemical systems: State of the art and prospects. *Journal of Power Sources.*, (2017) 356, 256-273.
- * This paper gives a detailed overview of bioelectrochemical approaches for CO₂ conversion.
- [7] O. Yehezkeli, R. Tel-Vered, S. Raichlin, I. Willner, Nano-engineered flavin-dependent glucose dehydrogenase/gold nanoparticle-modified electrodes for glucose sensing and biofuel cell applications. *ACS Nano.*, (2011) 5, 2385-2391.
- [8] A. J. Cracknell, A. Kylie, Vincent, and A. Fraser, Armstrong, Enzymes as Working or Inspirational Electrocatalysts for Fuel Cells and Electrolysis. *Chemical Reviews.*, (2008) 108 (7), 2439-2461.
- [9] A. Szczupak, Dan Kol-Kalman and L. Alfonta, A hybrid biocathode: surface display of O₂-reducing enzymes for microbial fuel cell applications. *Chem. Commun.*, (2012) 48, 49-51.
- [10] S. Srikanth, Y. Alvarez-Gallego, K. Vanbroekhoven, D. Pant, Enzymatic Electrosynthesis of Formic Acid through Carbon Dioxide Reduction in a Bioelectrochemical System: Effect of Immobilization and Carbonic Anhydrase Addition. *ChemPhysChem.*, (2017) 18, 3174-3181.
- * This paper shows a combination of enzymes namely, carbonic anhydrase for capture of CO₂ and its subsequent conversion by formate dehydrogenase.
- [11] Dominguez-Benetton, X., Srikanth, S., Satyawali, Y., Vanbroekhoven, K. and Pant, D., 2013. Enzymatic electrosynthesis: an overview on the progress in enzyme-electrodes for the production of electricity, fuels and chemicals. *J Microb Biochem Technol.*, (2013) 5, 6, p.2.
- [12] V. Flexer and N. Brun, N., Fundamentals of enzymatic electrochemical systems. *Funct Electrodes Enzym Microb Electrochem Syst.*, (2017) 3-50.
- [13] Blais R, Rogers P (2003) Process and apparatus for the treatment of carbon dioxide with carbonic anhydrase. US Patent 09/424,852:US6524843.

- [14] K. Habermuller, M. Mosbach, and W. Schuhmann., Electron-transfer mechanisms in amperometric biosensors. *Fresenius' Journal of Analytical Chemistry.*, (2000) 366 (6-7), 560-568.
- [15] W. Schuhmann., Amperometric enzyme biosensors based on optimized electron-transfer pathways and non-manual immobilization procedures. *Reviews in Molecular Biotechnology.*, (2002) 82(4), 425-441.
- [16] S. C. Barton, Enzyme catalysis in biological fuel cells: *Handbook of Fuel Cells.*, (2010) John Wiley & Sons, Ltd.
- [17] S. W. Xu, Y. Lu, J. Li, Z. Jiang, H. Wu, Preparation and Catalytic Properties of Novel Alginate–Silica–Dehydrogenase Hybrid Biocomposite Beads. *Ind. Eng. Chem. Res.*, (2006) 45, 4567-4573.
- [18] Q. Sun, Y. Jiang, Z. Jiang, L. Zhang, X. Sun, J. Li, Green and Efficient Conversion of CO₂ to Methanol by Biomimetic Coimmobilization of Three Dehydrogenases in Protamine-Templated Titania. *Ind. Eng. Chem. Res.*, (2009) 48, 4210-4215.
- [19] P.C. Sahoo, M. Kumar, A. Singh, M.P Singh, S.K Puri, S.S.V Ramakumar, Accelerated CO₂ capture in hybrid solvent using co-immobilized enzyme/complex on a hetero-functionalized support. *J CO₂ Util.*, (2017) 21, 77-81.
- [20] P. C. Sahoo, M. Kumar, A. Singh, M.P. Singh, S.K Puri., Biocatalyzed Accelerated Post-combustion CO₂ Capture and Stripping in Monoethanolamine. *Energy and Fuels.*, (2017) 31, 11007-11012.
- [21] O. Alvizo, L.J. Nguyen, C.K Savile, J.A Bresson, S.L Lakhapatri, E.O.P Solis, R.J Fox, J.M Broering, M.R Benoit, S.A Zimmerman, S.J Novick, J. Liang, J.J Lalonde., Directed evolution of an ultrastable carbonic anhydrase for highly efficient carbon capture from flue gas. *Proc Natl Acad Sci.*, (2014) 111, 16436–16441.
- [22] P. Majumdar, M.K. Bera, D. Pant, S. Patra, Enzymatic Electrocatalysis of CO₂ Reduction. In: Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Edition: 1, K. Wandelt, Victor Climent (Editors), (2018) Pp. 577-589. Elsevier. ISBN: 978-0-12-409547-2.
- [23] S.C Barton SC. Enzyme catalysis in biological fuel cells: Handbook of Fuel Cells., (2010) John Wiley & Sons, Ltd.
- [24] L. Zhang, J. Ong, J. Liu, S.F.Y Li., Enzymatic electrosynthesis of formate from CO₂ reduction in a hybrid biofuel cell system. *Renew. Energy.*, (2017) 108, 581-588.
- [25] M. Lienemann, J. S. Deutzmann, R. D. Milton, M. Sahin, A. M. Spormann, Mediator-free enzymatic electrosynthesis of formate by the *Methanococcus maripaludis* heterodisulfide reductase supercomplex. *Bioresour. Technol.*, (2018) 254, 278-283.
- [26] S. Srikanth, M. Maesen, X. Dominguez-Benetton, K. Vanbroekhoven, and D. Pant., Enzymatic electrosynthesis of formate through CO₂ sequestration/reduction in a bioelectrochemical system (BES). *Bioresource technology.*, (2014) 165,350-354.
- [27] E-G. Choi, Y. J. Yeon, K. Min, and Y. H. Kim. Communication—CO₂ Reduction to Formate: An Electro-Enzymatic Approach Using a Formate Dehydrogenase from *Rhodobacter capsulatus*. *Journal of The Electrochemical Society.*, (2018)165 (9) H446-H448.
- [28] N. Nemestóthy, P. Bakonyi, Z. Németh, K. Bélafi-Bakó, Evaluation of pectin-reinforced supported liquid membranes containing carbonic anhydrase: The role of ionic liquid on enzyme stability and CO₂ separation performance. *J. CO₂ Util.*, (2017) 24, 59-63.
- [29] C. Jin, S. Zhang, Z. Zhang, and Y. Chen. Mimic Carbonic Anhydrase Using Metal-Organic Frameworks for CO₂ Capture and Conversion. *Inorg. Chem.*, (2018) 57, 2169-2174.

- [30] V. C.-C. Wang, S. W. Ragsdale, F. A. Armstrong, Investigations of two bidirectional carbon monoxide dehydrogenases from *Carboxydotherrnus hydrogenofornans* by protein film electrochemistry. *ChemBioChem.*, (2013) 14, 1845–1851
- [31]. H. A. Hansen, J. B. Varley, A. A. Peterson, J. K. Norskov, Understanding Trends in the Electrocatalytic Activity of Metals and Enzymes for CO₂ Reduction to CO. *J. Phys. Chem. Lett.*, (2013) 4, 388–392.
- [32] Y. Amao, N. Shuto, *Res. Chem. Intermed.*, (2014) 40, 3267-3276.
- [33] P. K. Addo, R. L. Arechederra, A. Waheed, J. D. Shoemaker, W. S. Sly, S. D. Minter, Electrochem. Methanol Production via Bioelectrocatalytic Reduction of Carbon Dioxide: Role of Carbonic Anhydrase in Improving Electrode Performance. *Solid-State Lett.*, (2011), 14, E9–E13.
- [34] A. Basseghoda, C. Madden, D. W. Wakerley, E. Reisner, J. Hirst, Reversible interconversion of CO₂ and formate by a molybdenum-containing formate dehydrogenase. *J. Am. Chem. Soc.*, (2014) 136, 15473–15476.
- * A molybdenum-containing formate dehydrogenase H from the model organism *Escherichia coli* (EcFDH-H) was described with specific electrocatalytic CO₂ reduction.
- [35] M. Yuan, S. Sahin, R. Cai, S. Abdellaoui, D. P. Hickey, S. D. Minter, and R. D. Milton. Creating a low-potential redox polymer for efficient electroenzymatic CO₂ reduction. *Angew. Chem.*, (2018) 130, 6692 -6696.
- [36] S. Schlager, H. Neugebauer, M. Haberbauer, G. Hinterberger, N. S. Sariciftci, Direct Electrochemical Addressing of Immobilized Alcohol Dehydrogenase for the Heterogeneous Bioelectrocatalytic Reduction of Butyraldehyde to Butanol. *ChemCatChem.*, (2015) 7, 967-971.
- [37] S. Schlager, L. M. Dumitru, M. Haberbauer, A. Fuchsbauer, H. Neugebauer, D. Hiemetsberger, A. Wagner, E. Portenkirchner, N. S. Sariciftci, Electrochemical Reduction of Carbon Dioxide to Methanol by Direct Injection of Electrons into Immobilized Enzymes on a Modified Electrode. *ChemSusChem.*, (2016) 9, 631-635.
- * The immobilization of formate, formaldehyde, and alcohol dehydrogenases on one□and□the□same electrode for direct CO₂ reduction was demonstrated.
- [38] X. Chen, Y. Cao, F. Li, Y. Tian, and H. Song. Enzyme-assisted microbial electrosynthesis of Poly(3-hydroxybutyrate) via CO₂ bioreduction by engineered *Ralstonia eutropha*. *ACS Catal.*, (2018) 8, 4429-4437.

Highlights

Enzymatic electrosynthesis is a sustainable route for the conversion of CO₂ to chemical and fuels.

Formate dehydrogenase (FDH) and carbonic anhydrase (CA) are most studied enzymes.

Multi-enzyme systems are more efficient with high Faradic efficiency for conversion of CO₂ to product.