

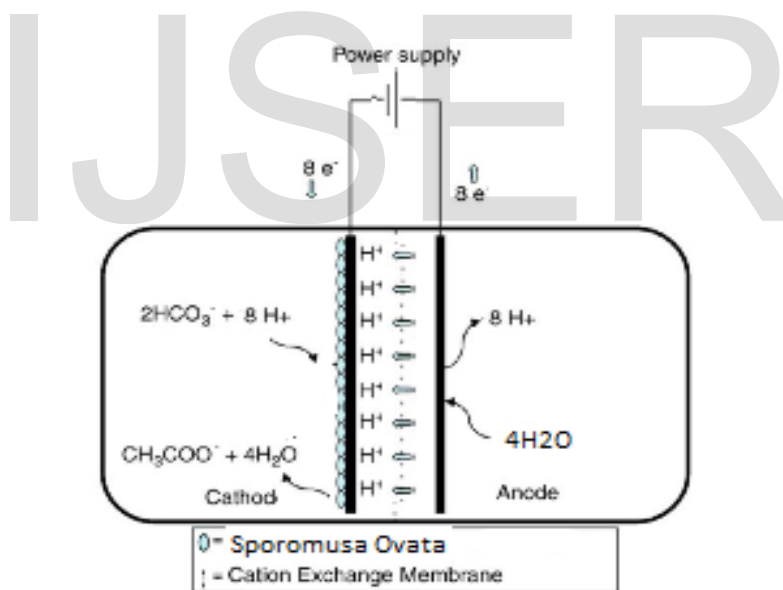
Bioelectrochemical Reduction of Carbon Dioxide to Organic Compounds

Robina Farooq^{1*}, Gagan Jabeen¹, Maria Siddique², Saleem Farooq Shaukat³, Saif Ur Rehman¹

Abstract—The present study demonstrates bioelectrochemical reduction of inorganic carbon dioxide to organic compounds using *Sporomusa Ovata* in a tube shaped bioelectrochemical cell (BEC). Among biosynthesized products acetate, ethanol, n-butyric acid and isopentanoic acid, 142.9 mg/L of acetate produced in 72 hours. This increase in acetate yield is attributed to improved parameters adopted during reactor design. Average bioelectrochemical acetate synthesis rate was found to be $1.3 \pm 0.67 \text{ mgL}^{-1} \text{ h}^{-1}$. Cyclic voltametric study confirmed redox activity of *S.Ovata* on poised biocathode. The percentage electron recovery as total organic compounds is found to be in the range of $84 \pm 13\%$ to $65 \pm 11\%$. The second major product is ethanol, formed by the conversion of acetaldehyde into ethanol. The presence of ethanol assumed to be due to electro activity and metabolic shift from acetate to ethanol in the biochemical-producing *S.Ovata* in BEC. The current research opens up the prospects of improving processes for bioelectrosynthesis of electron dense organic compounds from renewable energies and waste greenhouse gases instead of synthesizing them from non-renewable and energy rich compounds.

Index Terms—Anaerobic fixing of CO_2 , bioelectrochemical synthesis, living biocatalyst, columbic acetate recovery, fixing greenhouse gas, recovery of electrons, biofuels

GRAPHICAL ABSTRACT



Bioelectrochemical synthesis of organic compounds

HIGHLIGHTS

- Tube shaped bioelectrochemical cell (BEC) developed using novel combination of materials for cathode, anode and cation exchange membrane, used in bioelectrochemical harvesting of carbon dioxide.
- The amount of organic compounds and percentage recovery of electrons by *S.Ovata* is efficiently increased, attributed to improved reactor design and experimental conditions.
- Average rate of acetate synthesis was of $1.3 \pm 0.67 \text{ mgL}^{-1} \text{ h}^{-1}$.

- Ethanol formed due the conversion of acetaldehyde into ethanol due to electro activity and metabolic shift from acetate to ethanol.

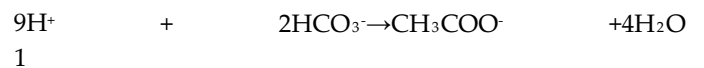
1. INTRODUCTION

Depleting fossil fuel resources, non-transportable renewable energies and use of agricultural land for biofuel production require sustainable solution of globally declared major challenges faced by humanity. The constant increase in greenhouse gases in air resulted climate change which may drag humanity to sewer droughts in some areas and flooding in others[1], resulting in food scarcity and water shortage problems. Solutions are required to fix greenhouse gases and convert them into value added products in the form of renewable chemicals.

Different processes are being used to harvest greenhouse gas CO₂ which are electro-catalytic reduction of CO₂[2], Enzymatic catalysis [3]and genetically modifications [4]. The electro-catalytically reduction of CO₂by using a range of different inorganic and organometallic catalysts are to fix atmospheric carbon dioxide to low chain organic compounds. These processesare plagued with poor thermodynamic efficiency, low current efficiency, low selectivity, slow kinetics, poor stability and high cost of metal catalysts, etc. The electrochemical fixing of CO₂ is also inundateddue to poisoning of electrodes, high cost of electrodes, use of hazardous solvents for concentrating CO₂, fouling of electrodes with byproducts, etc[5]. Use of enzymes as catalyst in electrochemical synthesis has limitations due to denaturing of enzymes.

Therefore, production of renewable bio-chemicals is presently fueling the debate on the sustainable synthesis of biofuels and bio-chemicals. The synthesis of biochemical by microbial electrochemical process offer many advantages over previous techniques. It can utilize low cost living biocatalysts in the form of acetogenic bacterial bio-film [6]having ability to auto activate themselves. Therefore, bioelectrochemical process is a low cost reduction of greenhouse gases to multicarbon organic compounds.

Reducing carbon dioxide to multicarboncovalent chemical bonds with renewable electricity has been identified as an attractive strategy[7, 8]. The organic compounds produced by current-driven microbial carbon dioxide reduction can then be distributed via existing infrastructure[9]. The microbial carbon dioxide reduction is possible by autotrophic bacteria. The autotrophic bacteria utilize carbondioxide or carbon monoxide during the synthesis of different organic compounds. These autotrophs, especially acetogens utilize Acetyl CoA pathway for converting C1 carbon into multicarbon compounds. The conversion of carbondioxide and hydrogen into acetate is shown in Equation 1[10].



The reductive acetyl-coenzyme A (CoA) pathway used by acetogenic bacteria where acetyl-CoA is synthesized from CO₂, H⁺ and electron donors and generate ATP by converting acetyl CoA to acetate. The key enzyme involved in this pathway is CO dehydrogenase. In this pathway, redox cofactors such as NADH or NADPH play an important role in cellular metabolism, while keeping cellular redox balance [11-13].

The genetic engineering of various enzymatic pathways to increase the NADH available to the cell is an effective way to increase the synthesis of desired products [14, 15]. An alternative approach is the use of bioelectrochemical technique for generating reduced NADH within the cell through interactions with an electrode in existing genetic code. Increasing the applied current to the cells would result in increased NADH which can change cell's NADH/NAD⁺ ratio. In the presence of CO₂, the product yields are relatively higher[16]. This pathway never adapt to oxic conditions as some of its enzymes, especially CO dehydrogenase /acetyl-CoA synthase are highly oxygen sensitive. Therefore, this pathway requires strict anaerobic conditions for the synthesis of target compounds[17].

The bioelectrochemical synthesis of organic compounds by *Sporomusa*, *Clostridium* and *Moorella* species is reported[9, 18-20]. The initial study shows the synthesis of organic acids including acetate using H type bio-electrochemical cell and recovery of electrons in the form of organic compounds or hydrogen[19]. However, it was found that

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the product yield and electrons recovery in organic compounds remained low.

Cathode in BEC where biofilm is developed plays a crucial role on the performance of bioelectrochemical cells. Graphite plate/rod, carbon cloth, carbon felt, carbon paper and, reticulated vitrified carbon graphite brush are being used as cathodic and anodic material. Carbon fiber type electrodes have gained importance due to their highly porous architecture. Recently, several new fiber electrodes were developed, such as carbon nanotube-textile, conductive nanowires network and electrospun carbon fiber mat, and had delivered a high current density [21]. However, the direct connection of such electrodes to external circuit remains a big challenge.

Metal materials, such as stainless steel (SS) materials, show excellent mechanical and electrical properties, low-cost, environmental stability and is easy to be shaped and connected. SS materials had been widely used as cathode or current collector of cathode in microbial electrochemical cells [22]. Carbon cloth is attached with stainless steel mesh in current study to get high porosity for residing bacteria and high conductivity and connectivity to external circuit.

Fixing inorganic CO_2 to organic compounds was undertaken using carbon cloth along with stainless steel as a cathode material by providing highly porous architecture of carbon cloth and stainless steel for being excellent mechanical and electrical properties. During current work, synthesized products are characterized where rate of synthesis and electrons recovery study was undertaken. The current approach was the use of whole organism as low cost living catalyst for reducing carbon dioxide into organic acids in microbial electrochemical cell (MEC).

2. MATERIAL AND METHODS

2.1 BIOELECTROCHEMICAL CELL AND METHODOLOGY

The bioelectrochemical synthesis of organic compounds was studied in a tube shaped bioelectrochemical cell (MEC), fabricated by non-corrosive and non-reactive poly methyl methacrylate material. The MEC consists of anode and cathode compartments, each having volume of 60cm^3 , separated by cation exchange membrane. Carbon cloth (C-Tex20, MAST Carbon International) along with stainless steel mesh plate is used as cathode and chromium plate (Aldrich/GF99465946) was selected as anode. Carbon cloth (C-Tex20) was selected due to high surface area of $1100\pm 150\text{m}^2/\text{g}$ and 3D morphology. It is used after rinsing

with distilled deionized water. Before the experiments, the stainless steel electrodes were cleaned with a 50–50% ethanol–acetone solution for 20 minutes under stirring to dissolve organic adsorbed species, then 20 min with a 2–20% fluorhydric–nitric acid solution to remove the oxide layer and were finally thoroughly rinsed in distilled water. Carbon cloth and stainless steel show same kinetic behavior for electron transfer [21], therefore both materials are placed together to enhance physical strength of carbon cloth by using stainless steel without effecting 3D surface area of carbon cloth for biofilm development. The proton exchange membrane (PEM), CMI-7000S was chosen due to its permeability to protons. The round shaped anode, cathodes and cation exchange membrane each having same area of 1257mm^2 were placed in MEC (Fig 1).

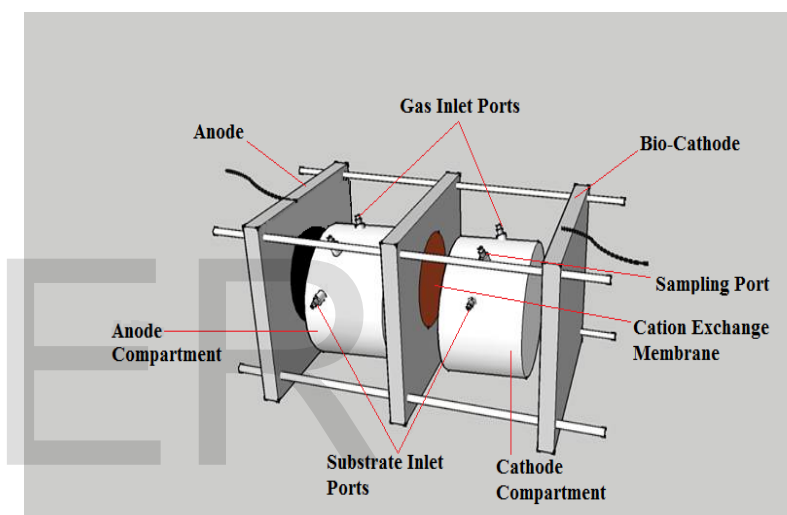


Fig 1: The Bioelectrochemical cell

Bacterial strain *Sporomusa Ovata* [23] was selected as a biocatalyst in current bioelectrochemical synthesis for its heterotrophic and exoelectrogenic characteristic [16]. *Sporomusa Ovata* is grown in DSMZ 311 culture media and is allowed to develop biofilm on carbon cloth placed in cathode compartment at $35\pm 2\text{ }^\circ\text{C}$. Vitamins and mineral solutions were added for ensuring healthy development of biofilm. The methodology for media preparation and uses of gas mixtures at different stages was as per described in literature [19].

Growth medium 311 for *S. Ovata* was prepared by dissolving all chemicals except bicarbonates, phosphate buffer, trace elements and vitamin solution in 500ml deionized water, pH of the medium was adjusted to 7.0 with 400ul of 3M HCl and boiled for five minutes. Medium was cooled in ice water under N_2/CO_2 (80/20) for 15 minutes and autoclaved for 20 minutes at 121°C . In autoclaved solution, trace element solution 1.0ml,

phosphate buffer solution 200ml, NaHCO₃ solution 80ml, NaHSeO₃ solution 10ml, vitamin solution 10ml, resazurin solution 2ml were added under anaerobic conditions for 1000ml media solution. Lysozyme (10ml/l) is added to suppress the growth of gram positive strains.

Sterilized, sealed serum bottles were first deoxygenated by passing nitrogen gas and medium of 10ml was added along with 2.0 ml culture of *S. Ovata* (DSM 2662) and were placed in the incubator inside the anaerobic chamber at 32°C. Bacterial growth was confirmed under microscope using gram staining technique. All stock solutions were kept at 4°C in refrigerator

Biofilm was allowed to grow on cathode in the presence of medium under H₂/CO₂ gas mixture whereas in anode compartment, medium was transferred excluding bacterial culture. In cathode compartment, medium was replaced several times to remove planktonic cells. The periodic removal of planktonic cells enhances the growth of biofilm on the cathode surface. Samples were taken after every 24 hours for organic compound analysis. Once concentration of acetate reached to 60mg/L, MEC was switched to CO₂:N₂ (20:80) gas mixture. Synthesis of organic compounds is not observed when electric current is discontinued. Organic compounds were not produced in controlled abiotic cell using sterilized medium 311, keeping all experimental conditions constant.

Experimental set up for BEC is shown in Fig 1 where electrons derived from anode are consumed by cathode at -400mV (versus SHE). Cathodic potential of 400mV is applied using DC power supply. Here, Ag/AgCl reference electrode is used, however all potentials are adjusted and reported according to SHE except in cyclic voltametric analysis. The number of electrons were calculated by; $1C = 1 \text{ Amp} \cdot \text{Second}$, $1C = 6.24 \times 10^{18}$ and $1\text{mol} = 6.02 \times 10^{23}$ electrons (96500Cmol^{-1}) [24]. The amount of hydrogen was determined using hydrogen detector (Model # SAH₂, Shi'An, China) at anode and was found to be less than 10mg/L throughout this study which is far less than the threshold concentration for acetogens to produce acetates. An important challenge to suppress the synthesis of hydrogen in the presence of CO₂ electrocatalysis, which is a dominant side reaction for CO₂ reduction from aqueous electrolytes. Strategies for suppressing hydrogen evolution is adopted by keeping higher CO₂ solubility in the media [2].

2.2 ANALYTICAL METHOD

2.2.1 CYCLIC VOLTAMMETRY (CV)

Cyclic voltammetric (CV) analysis was conducted using Reference 3000 Potentiostat (Gamary, Germany) to determine the redox potential of cathode in the presence and absence (control cell) of biofilm at scan rate of 10mV/s [25].

2.2.2 GC/MS ANALYSIS

Organic products were analyzed by head space solid-phase microextraction (HS-SPME) followed by gas chromatography (Agilent 7890A, US) equipped with Mass Spectrometer (5975C inert XLEI CI MSD detector).

For HS-SPME, Polydimethylsiloxane (100µm PDMS/CAR poly dimethyl siloxane/carboxene) coated fiber was utilized, liquid broth samples stored at -16°C were defrosted and 2 ml of them were pipetted out into 20ml screw-cap vials having silicon/PTFE septa. Internal standard 0.3 µl of 2-ethyl butyric acid and 0.75g of NaCl was added and mixed thoroughly. The pH was adjusted to 6.8 to 7 by 3 molar HCl and 0.15 M NaOH. Polydimethylsiloxane (100µm PDMS/CAR poly dimethyl siloxane/carboxene) coated fiber was utilized and stirred at 1000rpm and at 37 °C for 20 minutes. After extraction the fiber was desorbed directly to GC injector for 3 minutes. It was sufficient time for desorption of organic compounds under study and reinsertion of fiber after run without any carry-over [26, 27].

The quantification of organic products was performed by GCMS analysis using data acquisition system with computer software. An Agilent J&W (HP INNOWAX) open tubular capillary column (60m x 0.32mm x 0.32 µm). The injection volume of the sample for analysis was 1 µl. The column temperature was maintained at 70 °C for 2 minutes followed by ramping at rate of 40°C/min to 180°C maintained for 2 minutes and then at the rate of 20 °C/min. Temperature was increased to 200°C, holding the final temperature for 3 minutes. The injector and detector temperatures were 250 °C and 280 °C respectively. The GC system was operated in splitless mode with Helium as carrier gas at flow rate of 1.5ml/min. [28, 29].

MS was operated in a full scan mode (m/z 40-400) and in a selected ion monitoring (SIM) mode. Ions for detection of individual analyte VFAs in SIM mode were selected using the mass spectra of standards generated in SCAN mode [29]. The calibration of standard solution was performed by GC-MS by following the same set of conditions described above. Volatile acid mixture having C₂ to C₇ organic acids (Sigma Aldridge) was used as an external standard and

concentrations of unknown compounds are determined accordingly.

3. RESULTS AND DISCUSSIONS

3.1 BIO-ELECTROCHEMICAL SYNTHESIS OF ORGANIC ACIDS

The bio-electrochemical synthesis of organic acids by *S.Ovata* produced (Figure 2) 142.9 mg/L acetate in 72 hours. The small concentrations of ethanol, propionic acid, n-butanoic acid and iso-pentanoic acid were also detected during GC/MS analysis. The increase in yield is attributed due to the improved reactor design, kind of electrodes and cation exchange membrane. By placing carbon cloth attached with stainless steel at cathode provided conducive environment for the production of biofilm and chromium anode worked as a catalyst during electrolysis of water.

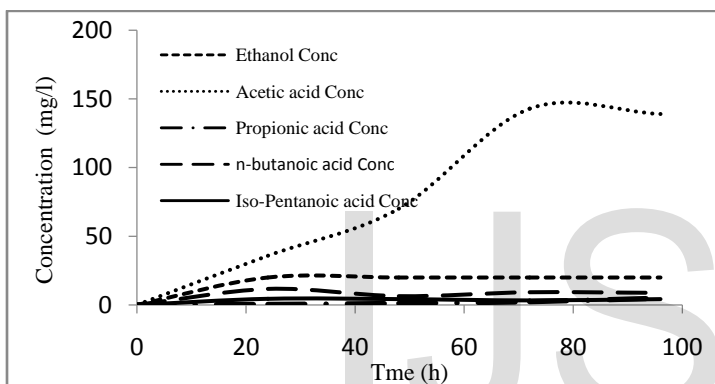


Fig 2: The bio-electrochemical synthesis of organic acids

The second major product is ethanol which may be formed due to the conversion of acetaldehyde into ethanol. A higher ratio of reduced forms of intracellular pyridine nucleotides increase the production of alcoholic metabolites such as ethanol, acetone, and butanol. Therefore, it is likely that the relatively higher enzyme activity for ethanol production from acetyl-CoA and the higher intracellular redox state resulted in the synthesis of these organic compounds including ethanol [30]. This is supported by similar studies where electricity-driven metabolic shift occurred through direct electron uptake by electroactive heterotroph *Clostridium pasteurianum*. In this study, CO₂ is bioelectrochemically converted into lactate followed by butanol production. It was found that this shift is due to a metabolic response to consume more NADH by producing more reduced compounds. It was investigated that conversion of lactate to butanol apparently occurred in BES. Considering all points together, it can be suggested that acetate in BES likely plays an important role as an

intermediate metabolite, consuming electricity-derived reducing power and converting into ethanol.

Similarly the presence of ethanol in our results demonstrates the possibility of electro activity and metabolic shift in the biochemical-producing *S.Ovata* is opening up the possibility of efficient and enhanced production of electron-dense metabolites in BEC [25]. Further studies are required to investigate metabolic shift during bioelectrochemical reactions.

The bioelectrosynthesis of the organic compounds showed that *S.Ovata* has consumed electric current and reduced inorganic carbon dioxide into organic acids. The synthesis of acetyl-CoA from CO₂ and H⁺ involves the formation of the methyl and carbonyl precursors of acetyl-CoA. The condensation of the above two precursors produces acetic acid, ethanol, butyric acid, butanol, and cell mass [31] (Fuchs 2011) (Fuchs 2011) (Fuchs 2011).

During several steps of the metabolic process electrons are required to carry out reduction reactions. Enzyme hydrogenase utilizes hydrogen to produce electrons for the formation of ethyl alcohol, acetic acid, or cell mass. In the current approach, electrons are provided through an external circuit in the absence of hydrogen molecules to produce acetyl-CoA. In the acidogenic phase, acetyl-CoA is converted to acetate and this pathway is followed during the rapid growth phase. Butanol and butyric acid can also be produced by combining two molecules of acetyl-CoA to form butyl-CoA. Under optimum growth at pH 6.0-7.0 the acetic acid is the major product [23]. Therefore, further studies were based on acetate synthesis.

3.2 RATE OF BIOACETATE SYNTHESIS

The average rate of acetate synthesis in the combined bio-electrochemical process is observed to be 1.3±0.67 mg L⁻¹ h⁻¹ (Table A.1).

Table A. 1: The rates of acetate synthesis by bioelectrochemical process

Time (h)	Bio-electrochemical acetate Synthesis	
	Concentration (mg L ⁻¹)	Rate of synthesis (mg L ⁻¹ h ⁻¹)
24	35.8	1.49
48	70.0	1.46
72	142.9	1.98
96	139.0	1.45

This increased synthesis in the bioelectrochemical process can be due to an increase in NADH available to the cell by

externally supply of electrons to bacteria. The bioelectrochemical technique NADH increases within the cell through interactions with an electrode and changes cell's NADH/NAD⁺ ratio [8]. In the absence of electrons supply as in biochemical process, bacteria have to pump hydrogen gas in and then have to utilize its electrons ($H_2 \rightarrow 2H^+ + 2e^-$) during one additional step which is omitted in bio-electrochemical process. The constant supply of electrons and H⁺ ions for increased NADH/NAD⁺ ratio and reduction of one chemical reaction may be the reasons for increased acetate synthesis. During bioelectrochemical synthesis of organic compounds, cathode serve as electron donors to CO₂ at inner side of inner membrane or in cytoplasm of bacteria. This reduction of electron acceptor (CO₂) and protons consumption produce organic compounds. This consumption of protons develop proton gradient across inner membrane[10].

It is observed that bacterial colonies started producing acetate and reached to maximum in 72 hours and then there is a decline in trend of acetate synthesis which is considered to be due to utilization of acetate by bacteria itself. This shows that continuous acetate removal from media is essential otherwise it is consumed up for biomass growth which slough off from cathode and cannot contribute efficiently for the synthesis of organic compounds.

3.3 ELECTRONS RECOVERY IN ORGANIC COMPOUNDS

The Cyclic voltammetry was performed on both biocathode(BEC) and abiotic cathode (control cell) as working electrodes against Ag/AgCl reference electrode. *Sporomusa ovata* showed the significant electroactivity and current consumption with definite redox peaks during 48 hours of cultivation as compared to sterilized control cell (Fig 3).

The control cell without microbes did not show the preferred redox peaks when CV was conducted against the reference electrode. Due to the absence of electroactive compounds i.e. microbes, the current transferred through the circuit is limited because the acceptance of electron at cathode is minimum. When the fresh medium was re-filled in the cathode compartment, it retained the electrochemical activities due to the presence of electroactive catalysts. The current consumption was recovered again when potential was applied indicated the transfer of current from cathode to microbes without mediators.

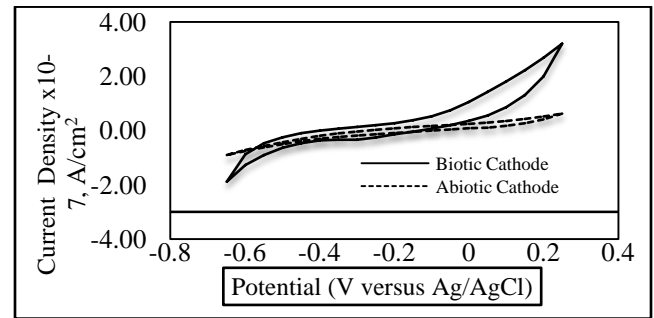


Fig 3: CV for *Sporomusa ovata* against standard Ag/AgCl reference electrode at scan rate of 10mV/s

The electrons consumed by *S. Ovata* and their recovery in organic compounds during bioelectrochemical process is confirmed and is shown in Figure 4a and 4b.

The electron recovery by organic compounds (ethanoic acid, ethanol, n-butanoic acid and iso-pantanoic acid, etc.) using *S.Ovata* is $84 \pm 13\%$ with the electrode area of 1257mm². The electron recovery of organic compounds is comparable to previous studies, which was found to be $86 \pm 21\%$ by *S.Ovata* with 1935.5mm² area of bio-electrode[19](Nevin, Woodard et al. 2010)(Nevin, Woodard et al. 2010)(Nevin, Woodard et al. 2010)[19]. The amount of organic compounds synthesis not only depends upon surface area exposed to biofilm but also on the kind of electrode. For a given geometric dimension, the highly textured carbon fiber anodes provide much more surface for microbial attachment than the flat surface. The results show that the percentage appearance of electrons in acetate by *S.Ovata* is $45 \pm 7\%$ where electron recovery is high in first 24hours and reached to $51 \pm 7\%$ in 72hours. The percentage efficiency of electron consumption by other researchers was reached to $48 \pm 6\%$ by using *S.Silvacetica* where surface area of bar electrodes used was 6500mm²[26].

This shows that electron recovery in current study is increased even at less electrode surface area exposed to biofilm while using carbon cloth along with stainless steel mesh. The recovery of electrons as organic compounds depends upon the rate of electron transfer. Enhancing the rate of electron transfer between electrodes to microorganisms is important to elucidate its mechanism. Electron transfer from electrode to cell is similar to microbial oxidation of reduced minerals or corrosion [32]. However, further research is required to find electron recovery behavior by adaptive evolution of microorganisms under selective experimental conditions. This approach may further enhance product yield.

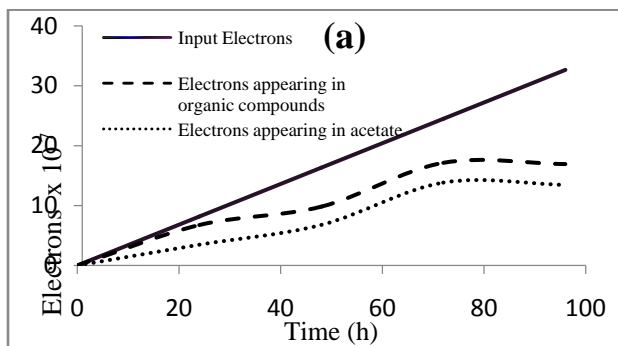


Fig 4a: The comparison of electrons input and its recovery in organic compounds

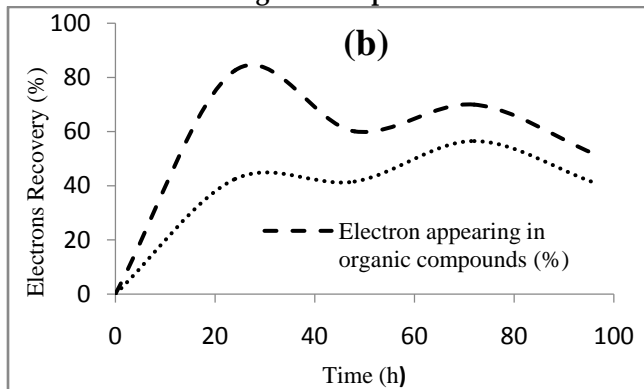


Fig 4b: percentage of electrons recovery in acetate and in all organic compounds

4. CONCLUSION

The current study for bioharvesting of CO₂ into renewable biochemicals using bioelectrochemical cell showed an increased acetate yield, rate of acetate synthesis and percentage recovery of electrons. Electrochemically active micro-organisms consumed part of the available energy to activate themselves and part of it for bioelectrochemical synthesis in an improved reactor specifications. The reduction of CO₂ in to organic compounds in bioelectrochemical process is similar to photosynthesis. Here extracellular compounds are formed rather than biomass which further require extraction process and hence increase the cost. Fixing of CO₂ not only can reduce global warming but can also decrease dependence on fossil fuels. Anaerobic bioelectrochemical fixing of greenhouse gas is important and there is a potential for synthesis of new biochemical. Attempts to bioelectrochemical fixing of CO₂ to biopolymers are underway.

REFERENCES

1. Ramanathan, V. and Y. Feng, Air pollution, greenhouse gases and climate change: Global and regional perspectives. *Atmospheric Environment*, 2009. 43(1): p. 37-50.
2. Shen, J., et al., Electrocatalytic reduction of carbon dioxide to

- carbon monoxide and methane at an immobilized cobalt protoporphyrin. *Nat Commun*, 2015. 6.
3. Shi, J., et al., Enzymatic conversion of carbon dioxide. *Chemical Society Reviews*, 2015. 44(17): p. 5981-6000.
4. CHIRKOV, A.M., Method of reutilization of carbon dioxide from emissions. 2013, Google Patents.
5. Appel, A.M., et al., Frontiers, opportunities, and challenges in biochemical and chemical catalysis of CO₂ fixation. *Chem Rev*, 2013. 113(8): p. 6621-58.
6. Du, Z., H. Li, and T. Gu, A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnol Adv*, 2007. 25(5): p. 464-82.
7. Rosenbaum, M.A. and A.E. Franks, Microbial catalysis in bioelectrochemical technologies: status quo, challenges and perspectives. *Applied microbiology and biotechnology*, 2014. 98(2): p. 509-518.
8. Rabaey, K. and R.A. Rozendal, Microbial electrosynthesis - revisiting the electrical route for microbial production. *Nat Rev Microbiol*, 2010. 8(10): p. 706-16.
9. Nevin, K.P., et al., Microbial Electrosynthesis: Feeding Microbes Electricity To Convert Carbon Dioxide and Water to Multicarbon Extracellular Organic Compounds. *mBio*, 2010. 1(2): p. e00103-10.
10. Lovley, D.R., Powering microbes with electricity: direct electron transfer from electrodes to microbes. *Environmental Microbiology Reports*, 2011. 3(1): p. 27-35.
11. Heise, R., V. Müller, and G. Gottschalk, Acetogenesis and ATP synthesis in *Acetobacterium itwoodii* are coupled via a transmembrane primary sodium ion gradient. *FEMS Microbiology Letters*, 1993. 112(3): p. 261-267.
12. Fuchs, G., CO₂ fixation in acetogenic bacteria: Variations on a theme. *FEMS Microbiology Letters*, 1986. 39(3): p. 181-213.
13. Ragsdale, S.W., et al., The acetyl-CoA synthase from *Clostridium thermoaceticum*: from gene cluster to active-site metal clusters. *FEMS Microbiology Letters*, 1990. 87(3-4): p. 397-402.
14. Atsumi, S. and J.C. Liao, Metabolic engineering for advanced biofuels production from *Escherichia coli*. *Current opinion in biotechnology*, 2008. 19(5): p. 414-419.
15. Zhao, H. and W. Chen, Chemical biotechnology: microbial solutions to global change. *Current Opinion in Biotechnology*, 2008. 19(6): p. 541-543.
16. Lovley, D.R. and K.P. Nevin, A shift in the current: New applications and concepts for microbe-electrode electron exchange. *Current Opinion in Biotechnology*, 2011. 22(3): p. 441-448.
17. Ragsdale, S.W. and E. Pierce, Acetogenesis and the Wood-Ljungdahl pathway of CO₂ fixation. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 2008. 1784(12): p. 1873-1898.
18. Nevin, K.P., et al., Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Applied and environmental microbiology*, 2011. 77(9): p. 2882-2886.
19. Nevin, K.P., et al., Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *mBio*, 2010. 1(2): p. 4818-4827.
20. Giddings, C.G.S., et al., Simplifying microbial electrosynthesis reactor design. *Frontiers in Microbiology*, 2015. 6: p. 468.
21. Pocaznoi, D., et al., Stainless steel is a promising electrode

- material for anodes of microbial fuel cells. *Energy & Environmental Science*, 2012. 5(11): p. 9645-9652.
22. Dumas, C., et al., Marine microbial fuel cell: Use of stainless steel electrodes as anode and cathode materials. *Electrochimica Acta*, 2007. 53(2): p. 468-473.
 23. Möller, B., et al., *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov. *Archives of Microbiology*, 1984. 139(4): p. 388-396.
 24. Gregory, K.B., D.R. Bond, and D.R. Lovley, Graphite electrodes as electron donors for anaerobic respiration. *Environmental microbiology*, 2004. 6(6): p. 596-604.
 25. Choi, O., et al., Electricity-driven metabolic shift through direct electron uptake by electroactive heterotroph *Clostridium pasteurianum*. *Scientific reports*, 2014. 4.
 26. Wejnerowska, G. and J. Gaca, Application of Headspace Solid-Phase Microextraction for Determination of Chloro-Organic Compounds in Sewage Samples. *Toxicology Mechanisms and Methods*, 2008. 18(6): p. 543-550.
 27. Banel, A., M. Wasielewska, and B. Zygmunt, Application of headspace solid-phase microextraction followed by gas chromatography-mass spectrometry to determine short-chain alkane monocarboxylic acids in aqueous samples. *Analytical and Bioanalytical Chemistry*, 2011. 399(9): p. 3299-3303.
 28. Geng, X., et al., Determination of organic acids in the presence of inorganic anions by ion chromatography with suppressed conductivity detection. *Journal of Chromatography A*, 2008. 1192(1): p. 187-190.
 29. Banel, A. and B. Zygmunt, Application of gas chromatography-mass spectrometry preceded by solvent extraction to determine volatile fatty acids in wastewater of municipal, animal farm and landfill origin. *Water Sci Technol*, 2011. 63(4): p. 590-7.
 30. Sakai, S., et al., Ethanol production from H₂ and CO₂ by a newly isolated thermophilic bacterium, *Moorella* sp. HUC22-1. *Biotechnology letters*, 2004. 26(20): p. 1607-1612.
 31. Fuchs, G., Alternative Pathways of Carbon Dioxide Fixation: Insights into the Early Evolution of Life?, in *Annual Review of Microbiology*, Vol 65, S. Gottesman and C.S. Harwood, Editors. 2011, Annual Reviews: Palo Alto. p. 631-+.
 32. Thrash, J.C. and J.D. Coates, Review: direct and indirect electrical stimulation of microbial metabolism. *Environmental science & technology*, 2008. 42(11): p. 3921-3931.

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Author contributions

RF conceived and designed this study and wrote the manuscript including figures (1-4) and Supplementary Table 1. G.J. performed experiment, M.S. partially analyzed data; S.R and S. F. S. helped in writing discussions. All coauthors contributed equally to the work being described. All authors reviewed the manuscript.

Additional Information - Competing Financial Interests

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