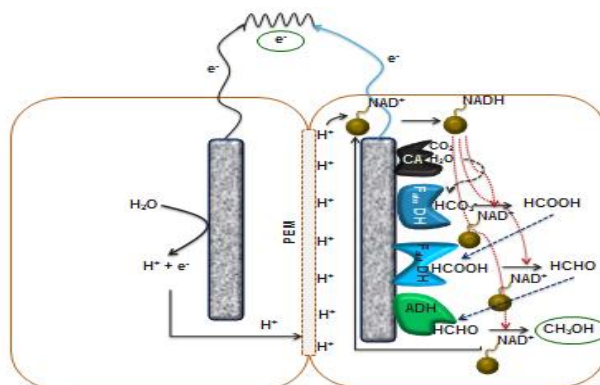


1. PUBLISHABLE SUMMARY

The current marie-curie project (ELECTROENZEQUEST) was proposed for the CO₂ reduction into methanol using dehydrogenases as cascade on electrode of bioelectrochemical system. Brief objectives include

- Immobilization of carbonic anhydrase (CA) on to the electrode and evaluation of its function as anode for electrogenesis in the fuel cell
- Bio-electrochemical characterization of the immobilized-CA electrode and optimization of various factors influencing the functional role of CA in CO₂ sequestration associated with electrogenesis
- Immobilization of Formate dehydrogenase (Fate DH), formaldehyde dehydrogenase (Fald DH) and alcohol dehydrogenase (Alc DH) on an electrode and its introduction as cathode in the operating CA fuel cell
- Optimization of factors affecting the fuel cell performance including the functional role of mediators such as NAD⁺, PQQ, etc., in mediating the electron transfer from the electrode to the substrate and in the conversion of CO₂ to methanol during cathodic reduction
- The bioprocess during fuel cell operation will be evaluated under optimum conditions for maximum product recovery with lowest possible energy inputs

Starting with these objectives, the project was completed successfully with all the objectives. Initially, we tried to study the feasibility of conversion of CO₂ into formic acid, using F_{ate}DH in free form.



Schematic representation of final developed BES for alcohol synthesis

After analyzing the results of the first experiment, we tried to immobilize the enzyme onto electrode F_{ate}DH. After several trials, we could successfully immobilize the enzyme onto graphite based VITO-CoRETM electrode. Then, we started working on immobilization of CA along with F_{ate}DH on same electrode to increase the formic acid productivity and CO₂ reduction. Further to that, we immobilized all three enzymes on a single electrode for production of methanol and CA was also added at later stage to increase the productivity. Detailed analysis of each experiment was done in comparison with controls. Immobilization in each experiment was done as follows

- » TBAB Modified Nafion solution was prepared for e⁻ transfer instead of H⁺ transfer
- » Electrode to be immobilized was taken and NR was polymerized on it
- » Known quantity of Enzyme (one or two) was dissolved in 100 µl of PBS (pH 7,4) in an eppendorf
- » Added with 50 µl of modified Nafion solution . Gently vortex for 5-10 sec
- » Place the electrode in a petri plate and pipette out the enzyme-nafion mixture on to the electrode and let it air dried
- » 100 mM NaNO₃ was poured on it after getting dried for equilibration



Enzyme immobilized electrode and the respective experimental set up

Different experiments were carried out using single enzyme and multiple enzymes. All the results were consolidated and presented as a Table 1.

Table 1: Comparative evaluation of obtained results with literature

Enzyme	Electrode	Conditions	Formic acid	Reference
Formate Dehydrogenase (F _{ate} DH)	Glassy carbon	PBS as Electrolyte (pH 7.4) Neutral red as mediator Applied potential, -800 mV Working volume, Not mentioned	32.207 mg/L (calculated based on conditions, 0.7 mM)	Addo et al., 2011
Formate Dehydrogenase (F _{ate} DH)	Cu electrode	PBS as Electrolyte (pH 7) [Cp*Rh(bpy)Cl] ⁺ as mediator Applied potential, -800 mV Working volume, 2.4 mL	Mentioned only % conversion, which is 12% at pH 7	Kim et al., 2014
Formate Dehydrogenase (F _{ate} DH)	Graphite plate	PBS as Electrolyte (pH 7.4) Neutral red as mediator Applied potential, -1 V Working volume, 10 mL	413 mg/L 9.37 mg/L CO ₂	Srikanth et al., 2014
Formate Dehydrogenase (F _{ate} DH)	Graphite based VITO-CoRE™	PBS as Electrolyte (pH 7.4) Neutral red as mediator Applied potential, -750 mV Working volume, 10 mL	544 mg/L 36.28 mg/L CO ₂	Unpublished
Formate Dehydrogenase and Carbonic anhydrase (F _{ate} DH+CA)	Graphite based VITO-CoRE™	PBS as Electrolyte (pH 7.4) Neutral red as mediator Applied potential, -750 mV Working volume, 10 mL	647 mg/L 43.13 mg/L CO ₂	Unpublished
Immobilized FateDH	Graphite based VITO-CoRE™	PBS as Electrolyte (pH 7.4) Neutral red as mediator Applied potential, -750 mV Working volume, 10 mL	497 mg/L 33.17 mg/L CO ₂	Unpublished
Immobilized FateDH+CA	Graphite based VITO-CoRE™	PBS as Electrolyte (pH 7.4) Neutral red as mediator Applied potential, -750 mV Working volume, 10 mL	623 mg/L 41.57 mg/L CO ₂	Unpublished

Research highlights

- Higher current densities due to the use of VITO based electrodes
- Co-factor recycling was achieved without any secondary system
- Immobilization brought stability in current densities
- Conversion efficiencies based on enzyme activity are good, However, the CO₂ supply can be reduced to increase the conversion rate
- CA addition helped a lot in increasing the reaction rates. Solubilized CO₂ can react more with the enzyme to form high product titre
- Specific activities of enzyme before and after the experiment weren't changed, which indicates the possibility of increasing the reaction time to get more product.

Further experiments were continued with three enzymes (FateDH, FaldDH and AlcDH) together on the electrode, where we have observed the ethanol production from CO₂ at a rate of 0.6 kg/m³/h. However, when we excluded the FaldDH also, there is no reduction in productivity of ethanol. It was surprising that we got ethanol instead of methanol but based on literature, it is also possible for the production of ethanol directly from formic acid, which is economically more viable. Further research is going on at host organization in this direction.