POLITECNICO DI TORINO

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Hybrid bio-electrochemical system for CO_2 reduction to valuable products

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Riassunto

Introduzione

Il riscaldamento globale è un problema mondiale; i suoi effetti coinvolgono livelli ambientali, di saluti, economici e politici. Una delle principali preoccupazioni in quanto al cambiamento climatico è la riduzione delle emissioni di CO_2. [1] Con questo obbiettivo alcune pratiche usate finora, da maggiori a minori contributi, sono l'implementazione di politiche di efficienza energetica, l'uso di fonti di energia rinnovabili, il cambiamento dall'uso del carbone per quello del gas, l'aumento della produzione di energia nucleare. Ancora l'uso della CO_2 gas come materia prima per prodotti di alto valore aggiunto ha una percentuale di contributo molto bassa in comparazione con gli altri [2], nel 2016, per esempio, da 24 Gt di CO_2 prodotti con i combustibili fossili, solo 200 Mt sono stati convertiti a prodotti di valore aggiunto, principalmente per la sintesi di carbonati e urea, solo qualche percentuale molta inferiore per produrre combustibili come il metanolo. [2]

La riduzione della concentrazione atmosferica della CO_2 è un foco di ricerca molto importante, ci sono studi legate alla cattura, allo stoccaggio e alla sua conversione e utilizzazione. Queste ultime possono farsi per processi fisici, chimici o biologici; a sua volta i metodi chimici possono essere di mineralizzazione, elettrochimici, termochimici e fotochimici. [2]. Fra i metodi a basse di processi biologici, l'uso di alghe sono state ampiamente investigate, e solo negli ultimi anni, l'uso di microorganismi ed enzimi.[3]

Il diossido di carbonio è il prodotto principale di tutte le combustioni; è una molecola molto stabile, quindi la sua conversione in qualsiasi molecola organica che includa il cambiamento del suo stato di ossidazione è molto difficile, en generale, sono processi endogeni ed endotermici. La Figura 1 presenta l'energia libera di formazione per diversi composti carboniosi, la non spontaneità della maggioranza delle reazioni può essere vista dal cambiamento positivo dalle energie di formazione dai prodotti con riferimento ai reattivi, Equazione 1. [2] Di questa forma, tutti gli approcci menzionati hanno in comune la necessita di catalizzatori per ridurre l'energia richiesta per far avvenire la trasformazione. Ogni tipo ha dei vantaggi e svantaggi, per esempio, i catalizzatori elettrochimici permettono di ottenere combustibile di trasporto, hanno una buona controllabilità e si sono trovati alcuni con buone efficienze e scalabilità. Invece, ci sono dei sistemi biologici, più amichevole con l'ambiente, molto selettivi e specifici, ma instabile, meno flessibile ai cambiamenti delle condizioni e più difficile da controllare.

$$\Delta G^0 = \sum_{Products} \Delta G_f^0 - \sum_{Reagents} \Delta G_f^0 \qquad \text{Equazione 1}$$

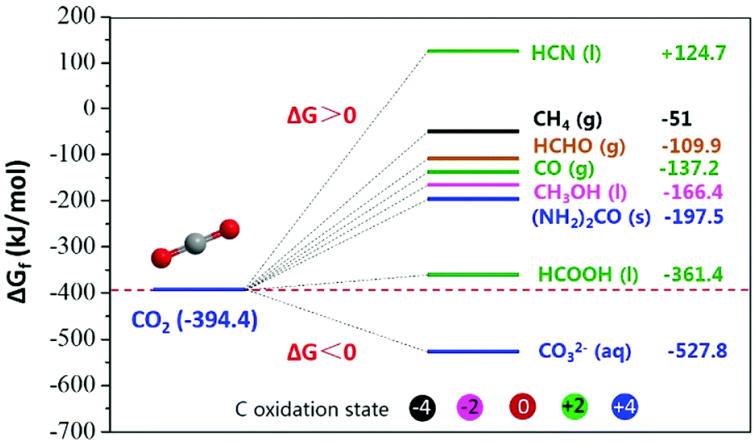

Figura 1.Valori delle energie libera di Gibbs standard per diversi composti carboniosi, gli stati di ossidazione del carbonio sono indicati d'accordo ai colori. Anche sono presentati i segni del cambiamento dell'energia libera di Gibbs dalla CO_2 alle altre molecole. Riprodotto da Ref [2].

Riduzione enzimatica

L'esempio di maggiore interesse per questo lavoro è la produzione di metanolo dalla reazione in cascata con tre enzimi, come è presentato nella Figura 2.

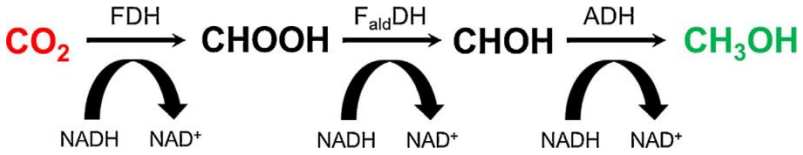

Figura 2. Cascata enzimatica per la trasformazione del diossido di carbonio in metanolo usando la Formato Deidrogenasi (FDH), la Formaldeide Deidrogenasi (FaldDH) e l'Alcol Deidrogenasi (ADH). Riprodotto da Ref [4].

Inizialmente, la CO_2 è soggetta a una reazione di idrogenazione per produrre formato attraverso l'enzima Formato Deidrogenasi (FDH), dopo, la Formaldeide Deidrogenasi (FaldDH) catalizza la reazione da formato fino ad aldeide, e finalmente, la formaldeide è ridotta a metanolo con l'aiuto dell'Alcol Deidrogenasi (ADH). In realtà, nei sistemi biologici questi enzimi fanno le reazioni inverse, cioè di ossidazione. Una parte molto importante della reazione è la nicotinammide adenina dinucleotide (NAD). Nella Figura 2 tre molecole di NADH sono ossidate a NAD^+, mentre che nella direzione di ossidazione di metanolo tre molecole NAD^+ sono ridotte. In natura il NAD^+ è rigenerato per altre reazione redox per ottenere di nuovo NAD^+, in cambio, per la produzione di metanolo l'uso di cofattore è un collo di bottiglia perchè un meccanismo secondario è necessario per non sacrificare il cofattore in ogni ciclo di reazione. È stato già dimostrato che fare la reazione da diossido di carbonio a metanolo è viabile ma ancora inefficiente, questa ha bisogno di lunghi tempi di reazione e presenta bassi valori di produzione. Uno dei maggiori problemi trovati è il secondo passaggio, perché la FaldDH è molto influenzata dal rapporto substrato-prodotto. [5]

Alcune definizioni importanti sono:

- **Attività enzimatica**: è una proprietà per misurare quanto lavoro catalitico può fare l'enzima. In termini cinetici, quanto se incrementa la velocità di reazione come conseguenza della riduzione della richiesta energetica. L'attività enzimatica viene così definita come la massima velocità di reazione (Vm). Per calcolarla si deve tenere in conto che la velocità di reazione dipende del tempo e della concentrazione di substrato, quindi deve essere calcolata come la velocità iniziale e con concentrazione di substrato cinque volte maggiori alla constante di Michaelis (Km: concentrazione di substrato dove V=Vm/2). [6] In avanti l'attività specifica è definita come si vede nella Equazione 2, dove una unità internazionale (UI) si riferisce a quanto enzima è necessaria per convertire una micromole di substrato o prodotto in un minuto a condizioni determinati. Per l'enzima ADH, l'attività specifica è misurata a pH 7 e 30ºC.

$$\text{Attività } specifica = \frac{UI}{mg\ enzima} = \frac{\mu mol\ NADH/min}{mg\ enzima} \qquad \text{Equazione 2}$$

- **Immobilizzazione:** è il processo per fissare la proteina in un supporto solido, serve per migliorare i principali svantaggi dei catalizzatori biologici, come i costi, la stabilità, l'isolamento e la purificazione. In questo lavoro sono stati valutati due metodi, l'immobilizzazione per legame covalente e per intrappolamento. La prima usa i gruppi amino dei residui di lisina e i gruppi aldeidi aggiunti con un previo processo di funzionalizzazione. La seconda, un metodo più semplice, usa un ricoprimento polimerico. I processi di immobilizzazione agiscono sulla stabilità incrementando la rigidità dell'enzima, ma l'attività può essere anche influenzata negativamente per blocco o disattivazione dei siti attivi. Anche il mass transfer viene influenzato secondo la porosità del materiale. È necessario definire due parametri per valutare l'idoneità del processo di immobilizzazione. [7][8]

- **Efficienza di immobilizzazione**: è l'estimazione della quantità di enzima supportata, ed è spresata in percentuale con riferimento alla quantità iniziale offerta al supporto.

- **Attività mantenuta:** Relazione di attività misurata nell'enzima immobilizzato e l'equivalenza di attività specifica come enzima libera della quantità di enzima data al supporto. [9]

Riduzione elettrochimica

Il fondamento teorico delle reazioni elettrochimiche è l'accoppiamento di due reazioni in due elettrodi diversi, una di riduzione e una di ossidazione, generando un flusso di elettroni tra di loro. Spontaneamente gli elettroni vanno del potenziale più basso a quello più alto.

Per generare la corrente inversa è necessario aggiungere un lavoro esterno usando una fonte di potenziale esterna, matematicamente questo si vede nel segno del potenziale totale della cella, positivo quando da energia e negativo quando l'energia deve essere alimentata al circuito. [10]

La riduzione di CO_2 richiede di elevati sovratensione, -1.9 V (vs RHE a pH 7) [11]. Questa energia può essere ridotta con l'uso di elettrocatalizzatori. Il parametro più importante per seguire le reazioni elettrochimiche è l'efficienza faradica. [2]

- Efficienza faradica: è espressa come il rapporto tra l'energia spenta per produrre una mole del prodotto desiderato e il totale di energia che passa attraverso del sistema. È una misura della selettività. Nell'Equazione 3, z è il numero degli elettroni, F è la constante di Faraday, n è il numero moli prodotti e Q è la corrente totale. [2]

$$\varepsilon_f = \frac{zFn}{Q} \qquad \text{Equazione 3}$$

Diversi metalli e ossidi sono stati studiati nella riduzione elettrochimica della CO_2; la Figura 3 riassume i metalli più utilizzati. La produzione di acido formico con superfici metalliche come Sn [12], [13], In, Pb and Hg [2], e la sintesi di monossido di carbonio in Au [4] o Ag [20] sono processi notevoli a causa della sua elevata selettività. Invece i catalizzatori a base di rame sono interessanti poiché si possono ottenere molti composti diversi [14], [15].

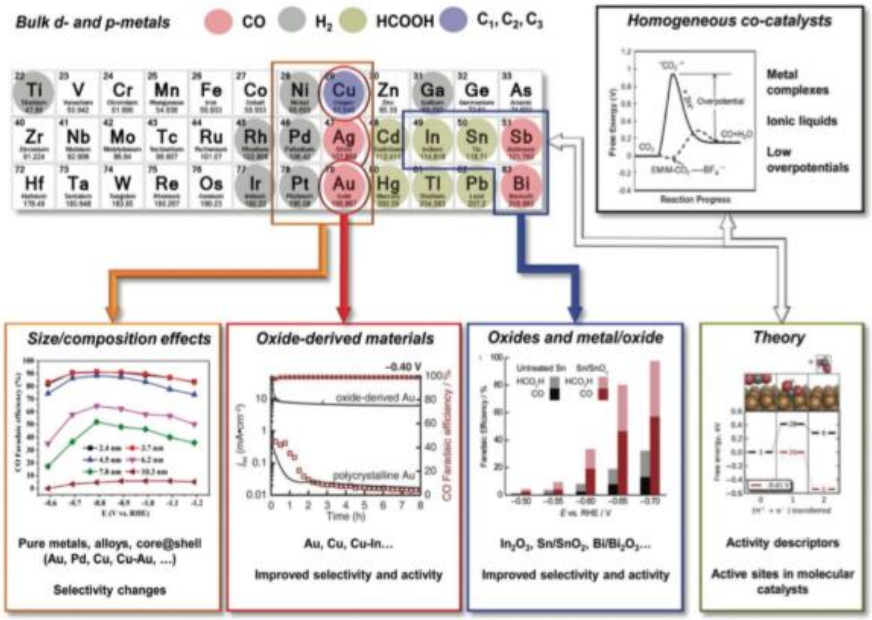

Figura 3. Principali elementi usati come catalizzatori elettrochimici, in bulk e ossidi. Anche sono indicati i principali prodotti e alcune caratteristiche del suo uso. Riprodotto da Rif [2]

Sistemi Ibridi

Alcuni approcci coinvolgono i microrganismi interi, questi possono prendere direttamente la CO_2 attraverso gli enzimi al suo interno e usarlo nei loro processi di trasferimento elettronico; altri approcci utilizzano gli enzimi isolati, in questo modo, i processi di purificazione sono più facili, si riducono i co-prodotti e permettono anche di scegliere i migliori enzimi e microrganismi, per ottimizzare le reazioni multi enzimatiche. Sistemi elettrochimici con i microorganismi sono le celle combustibile microbiche, le celle di elettrolisi microbiche e l'elettrosintesi microbica. [10]

Un esempio di sistema ibrido è mostrato nella Figura 4, la FDH è usata per ridurre il diossido di carbonio in formiato, mentre la rigenerazione del NADH è fatta con l'ossidazione di un complesso di rodio, che deve anche essere rigenerato, utilizzando una cella elettrochimica composta di un catodo di rame. Un risultato notevole di questo esperimento è che il sistema di rigenerazione sembra produrre anche formiato. [16]

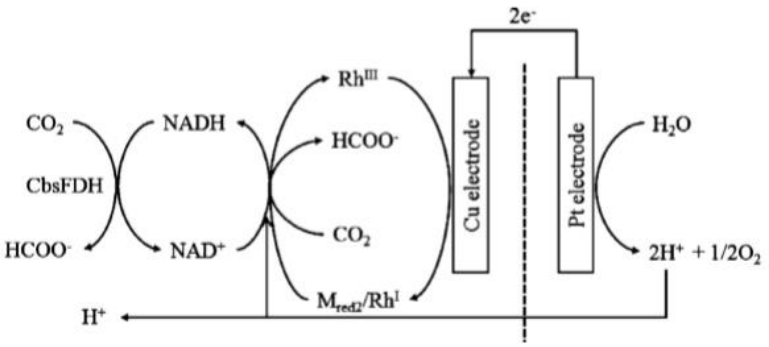

Figura 4. Sintesi di formiato dalla riduzione della CO_2 usando la Formiato Deidrogenasi di Candida Boidinii (CbsFDH) con un sistema elettrochimico usato per la riduzione di NAD^+. Un complesso di rodio è usato per la rigenerazione del cofattore e alla sua volta il complesso di rodio e rigenerato in un catodo di rame. Riprodotto da Ref [16].

Materiale supporto

Il feltro di carbonio ha un buon compromesso tra il suo prezzo, la porosità, la superficie e le sue proprietà conduttive; ciò significa un materiale più economico con un'elevata quantità di siti attivi, una buona resistenza meccanica e un'eccellente conduzione elettrica; Inoltre, il feltro di carbonio può essere funzionalizzato con gruppi specifici che rendono possibile il legame dell'enzima sul supporto. Invece, alcuni svantaggi sono la bassa bagnabilità e di conseguenza la bassa attività in soluzione acquosa. [17]

Obiettivo

Questo lavoro si centra nella trasformazione del diossido di carbonio in sostanze chimiche da alto valore aggiunto con una combinazione di tecniche chimiche e biologiche, un sistema ibrido elettrocatalitico-enzimatico. In questo modo vengono testati due sistemi principali. La ricerca fatta per Betjka et al [19] ha trovato risultati positivi sull'uso di ossido

di stagno nella produzione di acido formico, quindi nel primo sperimento, lo stesso elettrocatalizzatore è accoppiato con l'enzima Formiato Deidrogenasi, che è anche in grado di ridurre l'anidride carbonica in formiato. La riduzione di CO_2 nel sistema ibrido e in ogni catalizzatori per separato è valutata e paragonata con lo scopo di identificare i vantaggi di combinare entrambi sistema. Dopo, una reazione in sequenza è cercata, la CO_2 si spera sia ridotta con il rame, che in studi precedenti, ha presentato produzione di aldeide, e un secondo passaggio di reazione si fa con l'Alcol Deidrogenasi, che può sintetizzare alcoli dalle aldeidi.

Metodi Sperimentali

Il lavoro è stato fatto in due fasi, prima si è fatta la caratterizzazione dell'enzima ADH. L'attività dell'enzima libera è stata misura nella reazione di ossidazione di etanolo, a pH 7.5 e temperatura di 30°C, seguendo l'incremento del NADH per metodo spettrofotometrico, la reazione è avvenuta direttamente nella cuvetta aggiungendo 1 ml di etanolo 250 mM, 0.025 ml di enzima (0.01 e 0.005 mg/ml) e 0.050 ml di cofattore 100 mM. La Equazione 4 è stata usata per determinare l'attività enzimatica, $\Delta A/\Delta t$ è la pendenza del cambiamento del NADH in funzione del tempo, ε è coefficiente di assorbimento molare, 4.77 μmol/abs*ml, Vtot è il volume di reazione, Venz è il volume di soluzione enzimatica e Fdil è il fattore di diluzione, 100 per 0.01 mg/ml e 200 for 0.005 mg/ml.

$$Attività\ enzimatica = \frac{\Delta A}{\Delta t} \cdot \frac{1}{\varepsilon} \cdot \frac{V_{tot}}{V_{enz}} \cdot F_{dil} \qquad \text{Equazione 4}$$

Due tipi di immobilizzazioni sono state studiate. Per la tecnica di legame covalente il feltro di carbonio deve essere funzionalizzato aggiungendo gruppi aldeide come segue: prima l'adizione di gruppi epossidici usando 3-Glycidyloxypropyl-trimethoxysilane per 5 ore a 105°C (3.7 e 0.5% v/v sono stati provati), a continuazione un'idrolisi con acido solforico (0.1 M, 60 ml/gCF) a 85°C per 2 ore, e finalmente un'ossidazione con periodato di sodio (0.1 M, 60 ml/gCF) a temperatura ambiente per due ore. La quantità di gruppi aldeide (N_{ald}) aggiunti è calcolata per metodi spettrofotometrici quantificando quanto periodato di sodio, è reagito. [18] [19]

L'immobilizzazione è stata fatta aggiungendo il supporto funzionalizzato alla soluzione con la quantità di enzima desiderata (80 ml di buffer bicarbonato con 0.5, 1 o 4 mg di enzima). Il pH deve essere di 10.05, la temperatura di 4°C, e si mette in agitazione. In questa forma avviene il legame Shiff che dopo diventa covalente con boroidruro di sodio (0.5 mg/ml). L'immobilizzazione è seguita durante la formazione del legame Shiff prendendo campioni del sopranatante, questi se analizzano con il metodo di misura di attività enzimatica e con il metodo standard di quantificazione di proteina Bradford, rispettivamente sono indicativi qualitativi e quantitativi di quanto enzima rimane ancora nella soluzione.

Il secondo metodo di immobilizzazione studiato è l'intrappolamento con Nafion. La soluzione di immobilizzazione si prepara con 3.5 ml di Nafion, 3.5 ml di isopropanolo come solvente 1 ml di soluzione buffer e 1 ml di soluzione enzimatica con la quantità enzimatica da immobilizzare. La soluzione si deposita goccia a goccia di forma uniforme e omogenea sul feltro di carbonio. Questo approccio non può essere monitorato e si assume che tutto l'enzima è stato aggiunto al supporto.

La quantificazione dell'attività enzimatica immobilizzata si è fatta con la Equazione 5. La pendenza $\Delta A/\Delta t$ è stata calcolata per due procedure diverse. Prima reazioni di ossidazione di etanolo, partendo da 10 mg di feltro di carbonio immobilizzato, 2 ml di etanolo, 0.1 ml di NAD^+ e 0.05 ml di soluzione buffer, sono state fatte fermandoli a diversi tempi, il surnatante di ogni test è stato misurato per metodo spettrofotometrico prendendo un valore fisso di assorbanza, così si sono diagrammati i valori di assorbanza in funzione del tempo di reazione. Nel secondo metodo una sola reazione partendo da 0.1 g di supporto immobilizzato è stata fatta, tutti i reattivi si sono aggiunti nella stessa proporzione in referenza alla quantità del feltro di carbonio che nell'approccio 1, si sono pressi campioni del sopranatante ogni minuto per valutarli nello spettrofotometro e diagrammare l'assorbanza in funzione del tempo.

$$Enzyme\ activity = \frac{\Delta A}{\Delta t} \cdot \frac{1}{\varepsilon} \cdot \frac{V_{tot}}{mg_{enz}} \qquad \text{Equazione 5}$$

Finalmente i processi di immobilizzazione si sono valutati con due parametri, la efficienza di immobilizzazione, Equazione 6, per il metodo di legame covalente, e l'attività mantenuta, Equazione 7, per entrambi tecniche. Il primo è bassato nel calcolo di quantità di enzima fatto con il metodo di Bradford, C corrisponde a concentrazione, V a volume e gli indici i e f, rispettivamente al tempo iniziale e finale della formazione del legame Shiff.

$$IE = \frac{C_i V_i - C_f V_f}{C_i V_i} \qquad \text{Equazione 6}$$

$$\%Attività = \frac{Attività\ di\ enzima\ immobilizzato}{Attività\ di\ enzima\ libero} \qquad \text{Equazione 7}$$

La seconda fase del lavoro corrisponde alle prove elettrocatalitiche. Sulla deposizione degli elettrocatalizzatori inorganici si sono provato supporti con e senza trattamento termico (riportato in letteratura per incrementare la bagnabilità del materiale), la procedura per la modificazione del feltro di carbonio è stata una rampa di temperatura di 10°C per minuto fino a 500°C, con una tenuta di temperatura per cinque ore. Per tutte le deposizioni si è usata la tecnica di ricoprimento con spray. L'inchiostro di deposizione è stato fatto per

avere un carico di 3 mg di catalizzatori per g di supporto, considerando un'efficienza di 30% per la deposizione; il legante usato è stato il Nafion, in rapporto 72/28, il solvente l'isopropanolo, per una percentuale di solidi di 5 % w/w.

Si è usata una cella elettrochimica di due camere, il catodo con un volume di 20 ml e l'anodo di 40 ml, con una membrana (CMI 7000) per il trasporto di idrogeni tra di loro. L'elettrodo di lavoro è stato il catodo, un disco di feltro di carbonio di 1 cm di raggio e 0.7 cm di spessore con variazione di catalizzatori in ogni prova. Come contra elettrodo si è usato un foglio di platino e come elettrodo di referenza un elettrodo di argento/ cloruro di argento (Ag-AgCl). La soluzione di reazione è stata recirculata da un recipiente sigillato, dove sono gorgogliati i gas, anche dove si è messo l'uscita pero il cromatografo di gas (GC, Inficon Micro GC Fusion) per analisi in continuo. Il volume totale di reazione è stato di 60 ml e la soluzione è mantenuta a 30°C. Inoltre per la quantificazione dei prodotti liquidi, si sono pressi campione del volume di reazione per analizzarli per cromatografia liquida (HPLC, Shimadzu) e per cromatografo di gas accoppiato a spettrometro di massa (GC-MS, Model Clarus 580).

Il protocollo elettrochimico eseguito per tutti gli sperimenti si presenta nella Figura 5. Previo a ogni CV il sistema si è saturato per 30 minuti con il rispettivo gas. Si prendono 2 campioni, 1 ml ognuno, previo e dopo la CV con N_2, anche durante la CA a 1, 2 e 3 h. il flusso di CO_2 è stato fissato a 8.86 Nml/min.

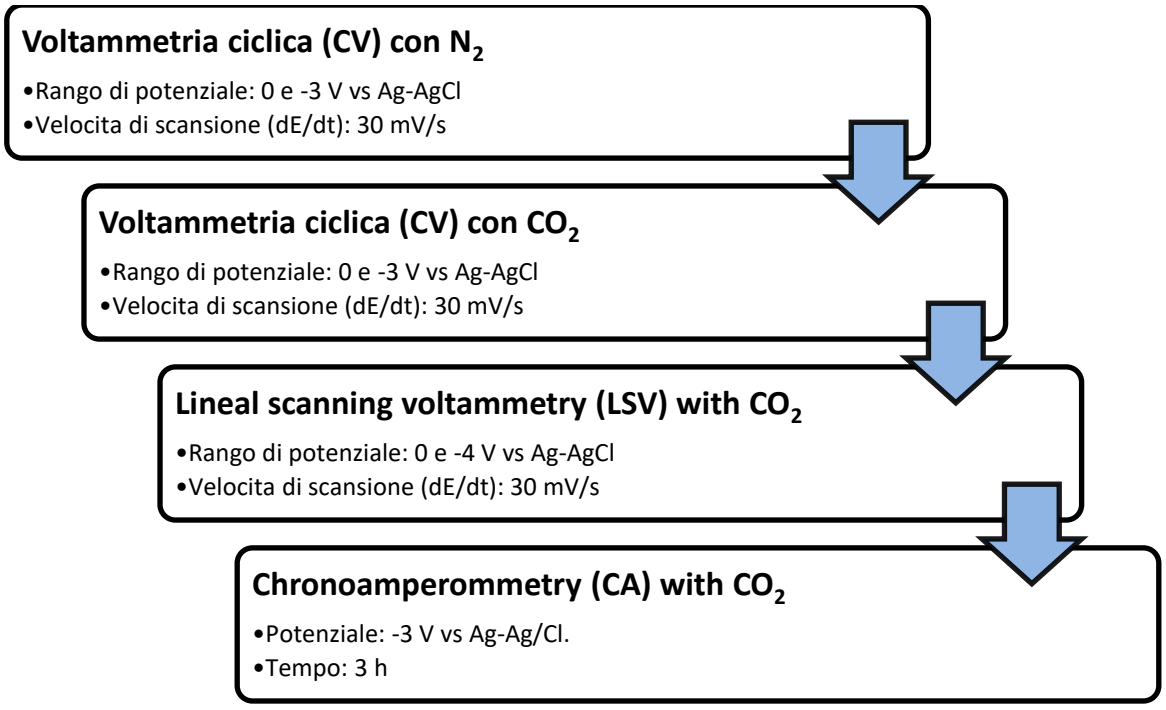

Figura 5. Protocollo elettrochimico usato nei test di attività catalitica. Le principali condizioni sono presentate.

La Tabella 1 riassume le specifiche dei catodi usati in ogni sperimento elettrocatalitico. In ogni camera della cella elettrochimica è stata usata una soluzione di bicarbonato di potassio 0.1 M. Inoltre per le prove con enzima anche è stato aggiunto il cofattore NADH, 1.5 ml dell'elettrolita si sono rimpiazzato con 1.5 ml di soluzione NADH 0.1 M.

Tabella 1. Test elettrochimici con le specifiche del catodo usato. Si indicano tipi di catalizzatori inorganici, di enzima e la forma aggiunta di questa ultima (immobilizzata o in soluzione).

Test	Pretrattamento del supporto	Catalizzatore	Enzima	Forma dell'enzima
1	No	SnO_2	Nessuno	Nessuno
2	Si	SnO_2	Nessuno	Nessuno
3	No	SnO_2	FDH	Soluzione (4mg/gCF/60ml)
4	No	SnO_2	FDH	Immobilizzato
5	No	Nessuno	FDH	Immobilizzato
6	No	Nessuno	Nessuno	Nessuno
7	No	Cu	Nessuno	Nessuno
8	No	Nessuno	ADH	Immobilizzato
9	No	Cu	ADH	Immobilizzato

Risultati

L'attività specifica enzimatica è stata calcolata per l'ossidazione di etanolo con l'enzima ADH a 30°C e pH 7.5. Si sono usati due campioni di una soluzione 1 mg/ml, per ognuno si sono fatte due diluzioni, e alla sua volta ogni diluzione si è valutata tre o quattro volte, in questa forma si è guarantito che il valore ottenuto come media dell'attività delle quattro diluzioni abbia una buona ripetibilità e che la procedura usata sia fidabile. Il valore trovato è di 228.99 UI/mg_{enz} ed è la referenza per i seguenti sperimenti.

Per l'immobilizzazione di legame covalente si sono usati pezzi di feltro di carbonio soggetti a una funzionalizzazione preliminare, per aggiungere i gruppi aldeidici. Due funzionalizzazione diverse sono state fatte con concentrazioni differenti del reattivo GPTMS, 167.5 mM e 22.64 mM, per i quali si sono quantificati rispettivamente, 1.304 e 1.895 mmol di aldeidi (CHO) per ogni g di supporto. Anche se la quantità di reattivo nel primo caso è maggiore, per circa 45%, è evidente che non c'è un incremento lineare del numero di molecole CHO, non è arrivato neanche al doppio. Tuttavia, entrambi materiale sono stati usati per fare immobilizzazione con l'enzima ADH, così da valutare se a maggior numero di gruppi aldeidici ci sono maggiori efficienze di immobilizzazione e migliori valori di attività di enzima immobilizzata.

Le efficienze di alcuni sperimenti sono riportate nella Tabella 2. Gli sperimenti Exp 2 ed Exp 3 sono stati fatti nel feltro di carbonio con minore contenuto di gruppi aldeidici, quindi ha senso avere negli altri maggiori rendimenti. Un'altra osservazione importante è che il carico di enzima desiderato negli sperimenti Exp 4, Exp 5 ed Exp 6 è stato di 4 mg/gCF, invece per lo sperimento Exp 3 è stato di 3 mg/gCF, e finalmente, per Exp 2 se cercava un carico di 0.5mg/gCF, quindi si evidenza che a maggiore quantità di enzima il processo di immobilizzazione ha migliori risultati, questo si è visto anche nelle procedure in laboratorio, visto che per la scala di lavoro la pesatura delle quantità necessarie d'enzima è stata una difficoltà.

Tabella 2. Risultati degli sperimenti di immobilizzazione, sono riportati il carico di enzima, l'efficienza di immobilizzazione e l'attività offerta al supporto

		Exp 2	Exp 3	Exp 4	Exp 5	Exp 6
Enzyme Loading	mg/gCF	0.120	0.651	3.285	0.763	0.813
IE	%	31.586	45.883	76.855	69.139	57.268
Activity offered	UI/gCF	27.587	149.134	752.353	174.785	186.141

La Figura 6 è un esempio dei risultati visti nei monitoraggi dei processi di immobilizzazione. Il parametro "slope" corrisponde al cambiamento di assorbanza (rappresentativo della concentrazione di NADH) in funzione di tempo, ottenuto al usare il campione come soluzione enzimatica per fare l'ossidazione di etanolo, in questo senso, a minore quantità di enzima rimanente nella soluzione di immobilizzazione (per trasferimento al supporto) minore evidenza di ossidazione, per conseguenza, minore produzione di NADH e finalmente si spera un minore "slope". Questo test corrisponde all'Exp 4, ma quasi tutti gli sperimenti hanno presentato lo stesso comportamento. Il campione di referenza (linea nera "Reference") permette vedere il processo di denaturalizzazione enzimatico generato per l'alto pH della soluzione di immobilizzazione. Invece, la curva "sample" corrisponde alla diminuzione dell'enzima nella soluzione per il trasferimento al supporto. Si può osservare un salto di pendenza da un valore maggiore a 0.6 Abs/min ("Reference" in tempo zero) a uno minore da 0.01 Abs/min ("Sample" a 20 min) in solo 20 minuti, questo significa che l'enzima si lega velocemente al feltro di carbonio. Da questi risultati si è individuato il tempo necessario per l'immobilizzazione, 40 minuti, già che si vede che dopo questo punto non si aggiunge più enzima al supporto. Inoltre c'è una diminuzione importante di pendenza nel "reference" da 40 a 60 minuti, quindi un'influenza importante del pH sull'enzima.

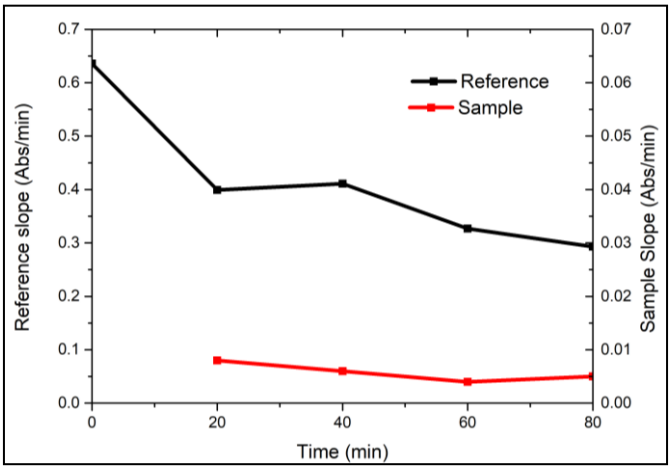

Figura 6. Seguimento di immobilizzazione di Exp 4, fatto con concentrazione di GPTMS 3.7% con carico enzimatico desiderato di 4 mg/g CF: Pendenza di assorbanza in funzione del tempo del surnatante della soluzione di immobilizzazione (sample) e della soluzione bianco con solo enzima solubile come controllo (reference).

I risultati delle misure di attività enzimatica per l'immobilizzazione di legame covalente evidenzia l'eterogeneità della distribuzione dell'enzima nel supporto, in fatto, non è stato possibile misurare un valore di attività perché il cambiamento della concentrazione di NADH alla fine delle reazioni non hanno presentato la dipendenza lineare con il tempo. Tuttavia si sono trovati tanti valori di assorbanza positivi che corroborano la presenza di NADH e, per conseguenza, di enzima immobilizzata in grado di realizzare la reazione.

Per l'immobilizzazione con Nafion non è possibile fare un monitoraggio della reazione, si assume che tutto l'enzima offerto al supporto viene legato. Il valore di attività offerto è di 694.47 UI/gCF. Contrario al metodo chimico, anche se si è osservata variabilità nei risultati, si sono riusciti a fare due misure diverse di attività, una per l'approccio di misure in discontinuo basato en diversi campioni di 10 mg di CF, e un'altra con il processo fatto prendendo tutti i campioni dalla stessa reazione bassata in 0.1 g di CF. Nei due casi si è ottenuta la stessa pendenza dell'assorbanza in funzione del tempo, cioè la stessa velocità di produzione di NADH, e per conseguente la stessa attività di enzima immobilizzata, 0.4 UI/gCF, cioè un'attività mantenuta di solo 0.058%. Ancora, questo risultato non si prende come indicativo di basso rendimento perché come si è detto prima, c'è stata anche molta variabilità nei risultati e il fatto di assumere che tutto viene immobilizzato sovrastima il valore di attività offerto. L'immobilizzazione con Nafion è stata scelta per fare gli elettrodi per le prove elettrochimiche perchè presenta più omogeneità che quella di legame covalente, è più semplice da fare, non richiede di pretrattare il feltro di carbonio, e anche, perché è più compatibile con il metodo di deposizione degli elettrocatalizzatori inorganici.

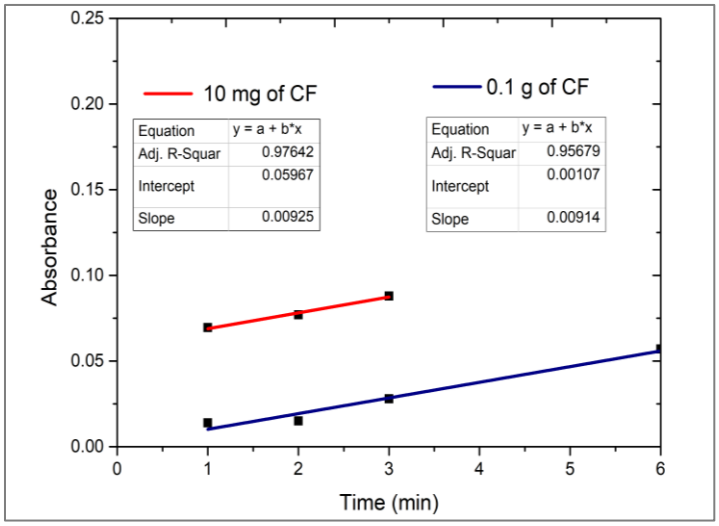

Figura 7. Assorbanza (rappresentativa della concentrazione di NADH) misurata a diversi tempi nell'ossidazione di etanolo con feltro di carbonio immobilizzato per il metodo di intrappolamento con Nafion (3 mg/gCF). Rossa: Test basato in 10 mg di supporto. Ogni punto corrisponde al valore medio di due sperimenti fermati ai rispettivi tempi di reazione. Blue: Test bassato in 0.1 g di supporto. Tutti I campioni sono stati pressi dello stesso set sperimentale.

I catalizzatori inorganici usati sono stati lo SnO_2 e il Cu. Entrambi sono stati depositati per il metodo di ricoprimento con spray con risultati migliori dal previsto, per lo SnO_2 si è osservato un rendimento circa al 50% di efficienza per i quattro elettrodi depositati, quindi anche ha mostrato una buona ripetibilità, invece per il Cu, le efficienze sono state tra il 30% e il 40%, questi valori più bassi sono coerenti con lo sviluppo sperimentale della tecnica, poiché è stato molto più difficile ottenere una dispersione omogenea del rame. Il pretrattamento termico nel feltro di carbonio, fatto per aumentar la sua bagnabilità e per tanto la sua affinità con l'inchiostro, non ha mostrato una differenza rilevante con riferimento all'elettrodo sopra il feltro di carbonio non trattato.

Per la quantificazione dei prodotti liquidi sono stati evidenziati problemi analitici e strumentali, principalmente a causa dell'interferenza del cofattore. Il NADH ha mostrato un alto segnale di risposta per il metodo HPLC usato, essendo soprapposto con i prodotti della riduzione di CO_2, per esempio con il formiato. Inoltre, per il metodo GC-MS dove i campioni vengono riscaldati in torno agli 80°C, il NADH presenta degradazione in molti prodotti, creando anche interferenza con i composti cercati come alcoli e aldeide. In conseguenza i risultati quantitativi dei test elettrochimici sono stati presentati in termini di efficienza faradica dell'idrogeno e di efficienza faradica mancante (differenza per completare il 100%) che si è assunto corrispondano alla riduzione di CO_2.

Nella Figura 8 si presentano le curve LSVs dei test degli elettrodi dei catalizzatori inorganici. L'elettrodo CF_{nt} (feltro di carbonio non trattato) ha presentato le maggiori correnti, con un potenziale onset di -0.8V vs Ag/AgCl, il CF_{nt}+SnO_2 ha avuto più attività dal CF_t+SnO_2 (Feltro di carbonio trattato con ossido di stanno) e, in quasi tutti i potenziali, CF_{nt}+Cu evidenzia una maggiore reazione rispetto agli elettrodi di stagno. In tutti i casi, gli

onset più pronunciati sono intorno ai -1,5 V vs Ag/AgCl, coincidente con il potenziale riportato in letteratura per la reazione evolutiva dell'idrogeno. Infatti, per i catalizzatori di ossido di stagno è difficile vedere gli altri cambiamenti di pendenza nel diagramma; su CF_t+SnO_2 c'è un piccolo cambiamento nella curva, intorno a -3,25 V vs Ag/AgCl, e in CF_{nt}+SnO_2 ce n'è un altro, circa a -2,3 V vs Ag/AgCl; i potenziali dopo di essere corretti con la resistenza dell'elettrolita corrispondono a -1.6 V e -1.7 V vs Ag/AgCl, valori di tensioni riportati in altre ricerche per la produzione di formato (tra -1.4 e -1.8 vs Ag/AgCl). [20]

I catalizzatori di rame sono stati ampiamente studiati nella riduzione della CO_2, ci sono ricerche, dove riportano al meno sedici diversi composti di carbonio generati dall'uso di rame in reazione di riduzione della CO_2. Per questo può essere più difficile specificare i potenziali di onset. Il valore più trovato in letteratura è -1,1 V vs Ag/AgCl, che potrebbe essere d'accordo con un leggero cambiamento ottenuto nella curva di CF_{nt}+Cu prima della riduzione di idrogeno. D'altra parte, ci sono anche documenti che presentano le riduzioni CO_2 circa a -1,5 V vs Ag/AgCl in questo caso, sarebbe sovrapposti con l'onset della RHE. [21] [22] [15].

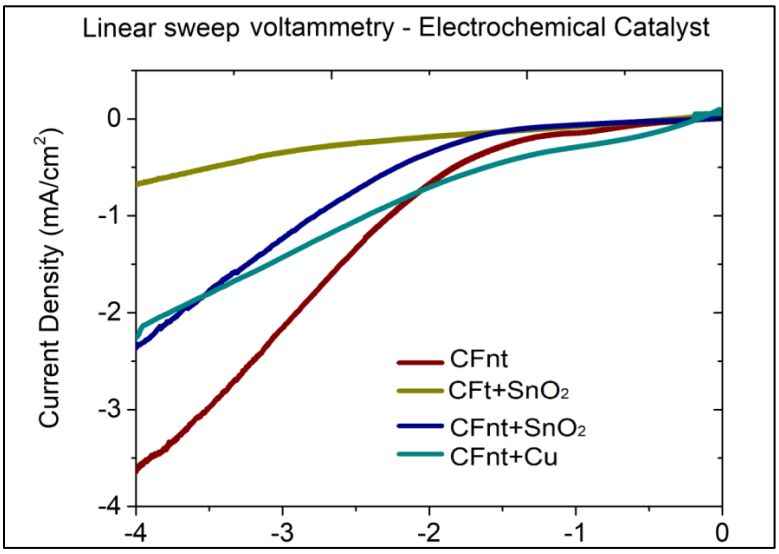

Figura 8. Voltammetria di scansione lineare in 4 elettrodi in soluzione acquose di $KHCO_3$ 0.1 M saturata con CO_2 a una velocità di scansione di 30 mVs^{-1}

Le CA e le analisi del GC per i catalizzatori inorganici si presentano nella Figura 9. Lo sperimento con l'elettrodo CF_{nt}+SnO_2 ha una buona stabilità con una densità di corrente nel test CA quasi costante intorno a -1 mA/cm^2, che si vede anche nell'analisi GC, dove prima c'è un tempo morto di circa 40 minuti e dopo la produzione di idrogeno arriva a un valore costante, vicino a 0,02 ml/min, che rimane fino al termine della reazione, 180 minuti, a questo punto sono state modellate due ore di degassato del reattore (linea tratteggiata), questo si è fatto per tutte le curve con base al degassato osservato nel test CF_{nt}+ADH. Dal CA e la GC anche si osserva concretamente che questo elettrodo ha un ritardo per arrivare

alle stesse condizioni stabili del CF_{nt}+SnO_2. Questo potrebbe corrispondere a problemi di trasferimento di massa; se avere una diversa distribuzione del catalizzatore all'interno del feltro di carbonio; se l'inchiostro del catalizzatore penetra più nel materiale, la CO_2 avrebbe bisogno di più tempo per arrivare alla superficie dell'ossido di stagno.

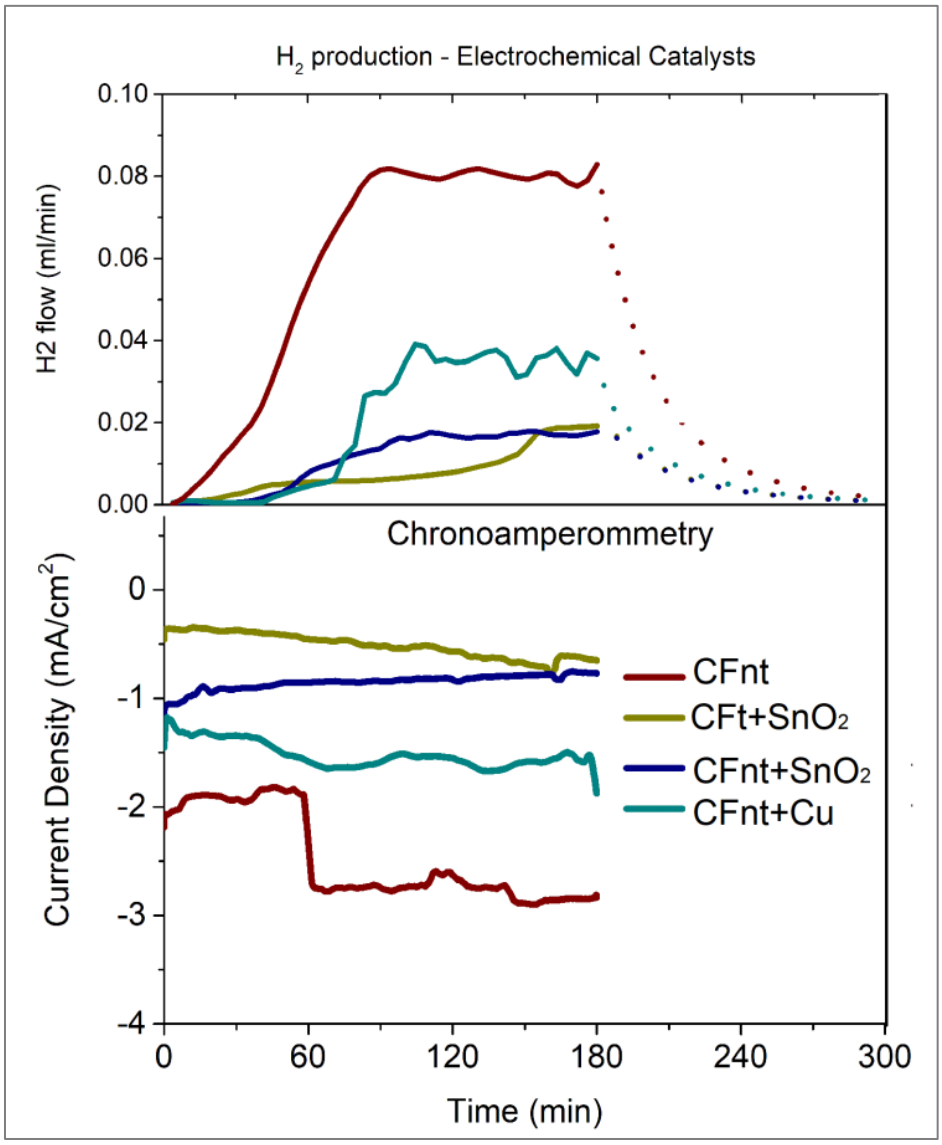

Figura 9. Flusso volumetrico di idrogeno (diagramma in alto) misurato con un gas cromatografo in continuo durante la cronoamperommetria (diagramma in basso) in 4 elettrodi in soluzione acquose di $KHCO_3$ 0.1 M saturata con CO_2 a -3 V vs Ag/AgCl. L'ultima parte del flusso di idrogeno (linea tratteggiata) è stata modellata, invece di misurata. La curva in grezzo delle misure del GC per il CFnt è anche indicato (Diagramma ausiliare in alto a destra).

La peggiore attività di riduzione è stata vista ne sistema CF_{nt}+SnO_2+FDH_{Sln}. Una risposta similare si osserva al paragonare lo sperimento con l'enzima, con il catalizzatore inorganico e con il sistema ibrido, in entrambi diagrammi di LSV, Figura 10, si osserva che il sistema ibrido ha dei valori di densità di corrente intermedie tra quelli dei catalizzatori inorganici e

quelli dei biocatalizzatori. Va ricordato che non si prevede che il Cu e l'ADH funzionino allo stesso modo di SnO_2 con FDH, invece di cercare la produzione dello stesso composto in entrambi i tipi di catalizzatori, si prevedeva una reazione sequenziale, prima riduzione di CO_2 ad aldeidi (sulla superficie del Cu) che fungeranno da substrato dell'ADH per la produzione di alcoli. Tuttavia, i risultati hanno indicato che anche se hanno meccanismi diversi, dovrebbe essere un vantaggio comune nell'uso di entrambi i tipi di catalizzatore con riferimento all'utilizzo degli elettrocatalizzatori classici.

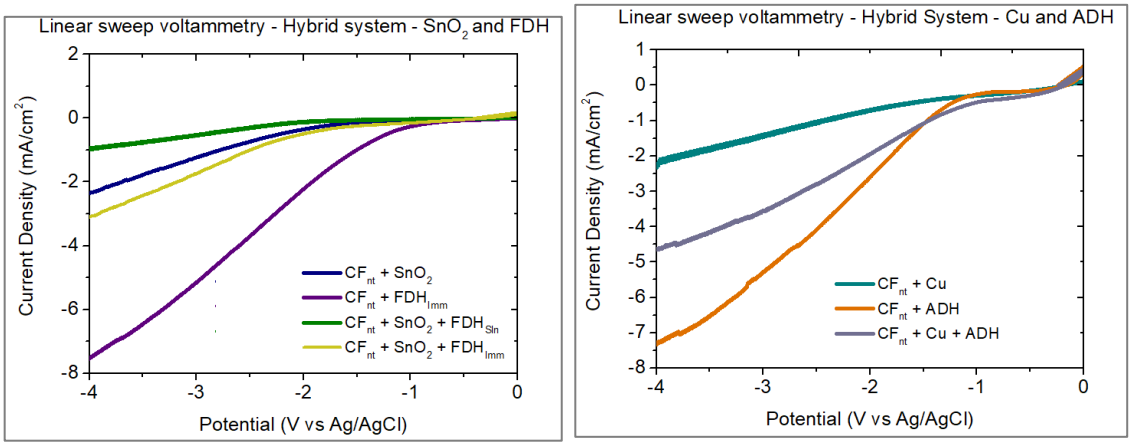

Figura 10. Voltammetria di scansione lineare in 4 elettrodi in soluzione acquose di KHCO3 0.1 M saturata con CO_2 a una velocità di scansione di 30 mVs-1

Nella Figura 11, la CA dell'elettrodo CF_{nt}+FDH ha mostrato una densità di corrente inferiore rispetto al valore indicato dall'LSV, inizia con una corrente più elevata, ma si osserva una forte caduta di attività dopo pochi minuti. Questo risultato è coerente con il comportamento dell'enzima, in cui all'inizio è presente in soluzione una concentrazione più elevata di cofattore, quindi è possibile che la reazione sia limitata dalla rigenerazione di NADH. In questo modo, anche se nel CV e nel LSV dell'elettrodo con l'enzima immobilizzato mostrava una migliore attività elettrochimica, le correnti più elevate sono state evidenziate con i campioni CF_{nt} + SnO_2 + FDH_{Imm}. Tuttavia, questo test ha anche presentato la maggiore produzione di flusso di idrogeno, pertanto sono state calcolate efficienze faradiche che verranno discusse di seguito per quantificare la reale attività catalitica. Va anche notato il comportamento simile della curva dell'idrogeno dell'elettrodo con solo l'enzima e con il sistema ibrido.

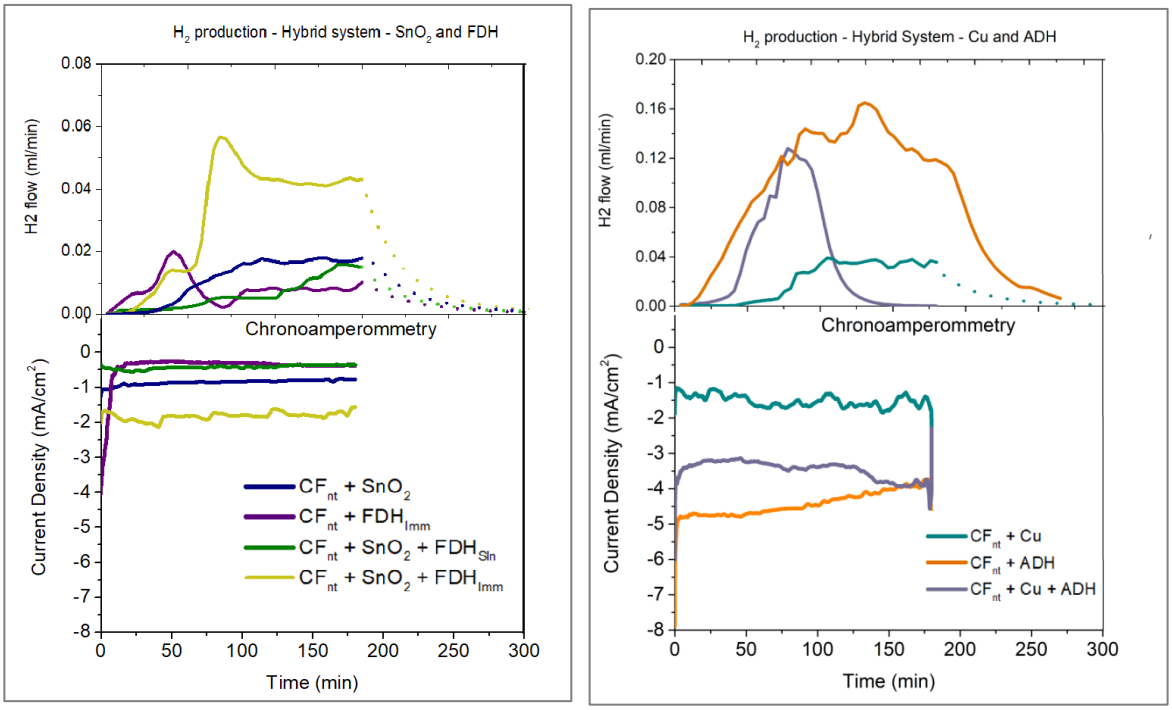

Figura 11. Flusso volumetrico di idrogeno (diagrammi in alto) misurato con un gas cromatografo in continuo durante le cronoamperommetria (diagrammi in basso) in 7 elettrodi in soluzione acquose di $KHCO_3$ 0.1 M saturata con CO_2 a -3 V vs Ag/AgCl. L'ultima parte del flusso di idrogeno (linea tratteggiata) è stata modellata, invece di misurata. La curva in grezzo delle misure del GC per il CFnt+FDH_{Imm} è anche indicato (Diagramma ausiliare in alto a destra).

Per l'esperimento CF_{nt}+FDH_{sln} la produzione di H_2 ha la particolarità di arrivare stato stazionario di CF_{nt}+SnO_2. Questo comportamento è stato osservato anche per l'ossido di stagno depositato sul materiale trattato. È stato proposto che per il trattamento siano state aumentate le limitazioni del problema di trasferimento di massa. Pertanto, una possibilità è che l'enzima in soluzione possa anche generare una sorta di problema di questo tipo.

La curva di produzione di H_2 per l'esperimento CF_{nt}+Cu+ADH è notevoli, circa 75 minuti è apparso un picco massimo di più o meno 0,12 ml / min, dopo è diminuito rapidamente e circa 150 minuti già tendeva a zero. Poiché la corrente di densità è rimasta quasi costante, questo fenomeno può essere una prova del funzionamento sequenziale dell'elettrodo e dell'enzima, in cui la prima parte corrisponde al lavoro del rame, che produce in parallelo idrogeno e alcuni prodotti a riduzione di CO_2 (tra cui alcune aldeidi), e alla fine l'azione dell'enzima che prende come substrati gli aldeide prodoti dal rame. Tuttavia, questa ipotesi dovrebbe essere confermata con la quantificazione dei prodotti e dovrebbe anche essere proposto un meccanismo logico in cui quando il biocatalizzatore funziona inibisce anche la reazione di HER.

Nel secondo sistema ibrido le densità di corrente concordano con le informazioni ottenute dalle curve degli LSV. Con l'elettrodo enzimatico ADH si verifica anche una rapida caduta iniziale di corrente, ma si è stabilizzata a circa -5 mA/cm^2, a seguito diminuisce

lentamentefino al valore di densità di corrente stabile dell'elettrodo ibrido. Chiaramente, l'ADH non può ridurre la CO_2, quindi la corrente osservata è concordante con l'alto flusso di H_2.

Sono state calcolate le efficienze faradiche, Equazione 3, queste sono riportate nella Figura 12; a causa della differenza nella scala delle FE% dell'idrogeno con la FE% acetone e isopropanolo sono stati utilizzati due assi, uno a sinistra per H_2 e uno a destra per gli altri due composti.

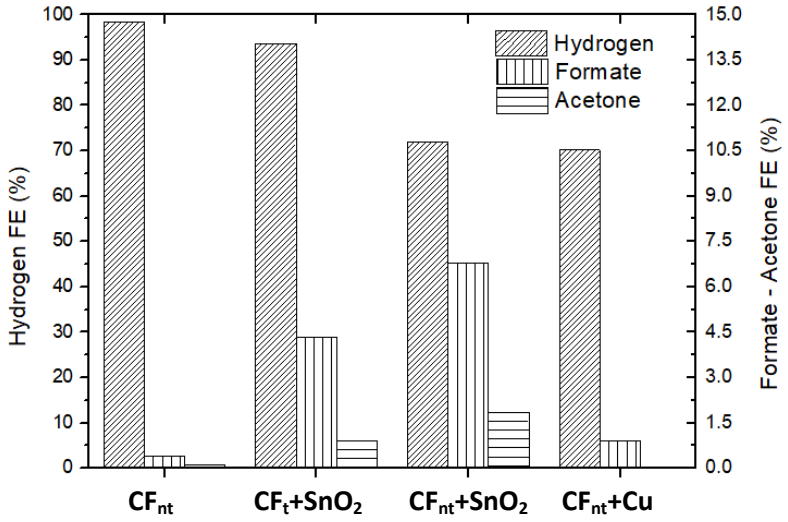

Figura 12. Efficienze faradiche per l'idrogeno (asse destro), il formato e l'acetone (asse sinistra) per 4 elettrodi: CFnt, CFt+SnO_2, CFnt+SnO_2 e CFnt+Cu.

Per il CF_{nt} si osserva che quasi l'intera reazione corrisponde principalmente alla RHE, FE = 98,4%, tuttavia, la produzione di formiato e acetone dal supporto, senza catalizzatori, è notevole, anche se hanno efficienze minore dell'1%. In futuro potrebbe essere studiata l'ottimizzazione della struttura di supporto e le deposizioni del catalizzatore per ottenere un sistema catalitico migliore. La riduzione della CO_2 è dimostrata in tutti i sistemi inorganici, negli elettrodi di ossidi di stagno con efficienze faradiche cinque e sette volte superiori rispetto al rame, rispettivamente per CF_t+SnO_2 e CF_{nt}+SnO_2.

Il comportamento molto migliore del CF_{nt}+SnO_2 può essere osservato perché CF_t + SnO_2 consuma il 22% in più di elettroni nella riduzione dell'idrogeno. In effetti, nel CF_{nt}+SnO_2 ci sono ancora circa il 20% della FE totale da quantificare. Il rame ha risultati di riduzione dell'idrogeno simili rispetto a CF_{nt}+SnO_2, ma va sottolineato che il tasso di produzione è inferiore perché il suo caricamento del catalizzatore è di 4,40 mg/cm^2 e quello di CF_{nt}+ SnO_2 è di 5,06 mg/cm^2. Per CF_{nt}+Cu non si è quantificato quasi nessun prodotto per la riduzione della CO_2, ma mancano da indiviuduare il 30% delle FE. In questo senso, è impotante dire che i metodi di analisi HPLC e GC-MS sono calibrati solo per determinate sostanze, quindi ci sono alcuni picchi che non sono stati ancora identificati. Inoltre, sono

stati richiesti un totale di 60 ml di reazione volumetrica nella cella elettrochimica per il ricircolo e il riscaldamento esterno, questo dominuisce le concentrazioni di prodotti e possono esserci composti carboniosi in concentrazioni inferiori ai limiti di rilevamento degli strumenti. I sistemi con enzimi hanno presentato l'ulteriore problema per quantificazioni liquide già detto ptima, l'interferenze del NADH. Il metodo è stato ottimizzato per separare i picchi, ma le nuove condizioni tecniche hanno prodotto anche maggiori limiti di rilevazione e quantificazione.

Nella Figura 13 l'elettrodo che presenta un'efficienza faradica dell'idrogeno inferiore e quindi, una maggiore attività di riduzione della CO_2, è il catalizzatore inorganico, CF_{nt}+SnO_2. È stato ipotizzato che l'enzima in soluzione, con peggiori prestazioni catalitiche, abbia creato una sorta di interferenza per la riduzione di CO_2 nell'elettrodo, questa idea può essere ampliata all'enzima immobilizzato, il CF_{nt}+SnO_2+FDH presenta maggiore densità di corrente ma anche una maggiore produzione di H_2. Questo può essere correlato all'immobilizzazione, ad esempio, per l'incremento nella quantità di Nafion che potrebbe anche agire come barriera per la CO_2. In studi futuri, Un altro parametro importante che può essere valutato in futuro è l'influenza della concentrazione di NADH, questo sarebbe utile per migliorare le tecniche analitiche e strumentali, ma ancora più importante, per capire meglio il funzionamento dell'enzima. Comunque un buon indicativo è che dovuto all'incremento di corrente, anche se l'efficienza per i prodotti della riduzione di CO_2 è minore, si otterrebbero maggiore quantità che negli altri sistemi.

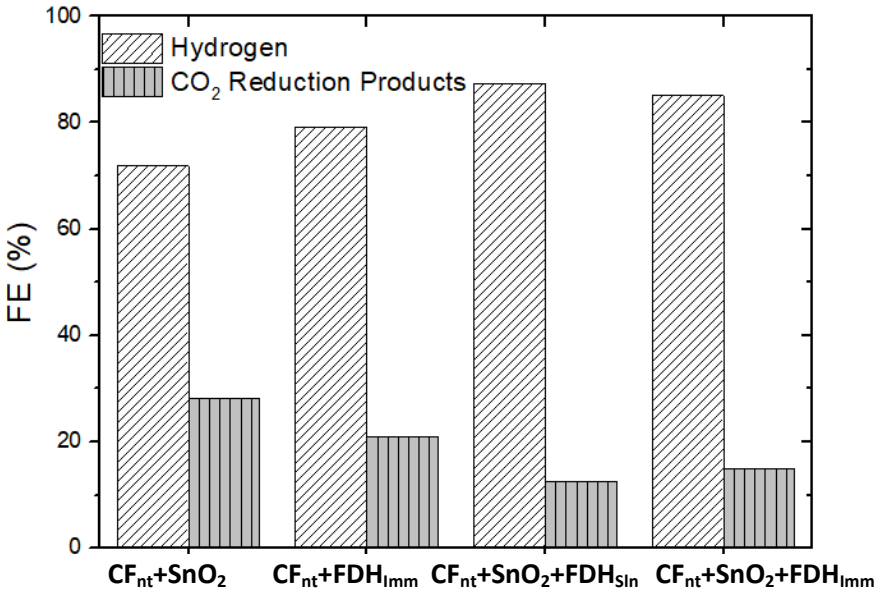

Figura 13. Efficienze faradiche per l'idrogeno e per i prodotti della riduzione della CO_2, per 4 elettrodi: CFnt+SnO_2, CFnt+FDH, CFnt+SnO_2+FDH_{Sln} e CFnt+ SnO_2+FDH_{Imm}.

Infine, il secondo sistema ibrido ha mostrato risultati molto promettenti. Con l'elettrodo CF_{nt}+ADH, nelle CA, si sono visti le correnti più elevate di tutti gli esperimenti sviluppati, ma come è stato previsto, tutto corrisponde all'HER. Poiché l'ADH non produce in alcun modo idrogeno, significa che l'enzima genera un importante aumento di corrente nel supporto, che è dove avviene la reazione. I migliori risultati di tutti i test possono essere attribuiti al sistema ibrido di Cu e ADH, più della metà dell'efficienza faradica dovrebbe corrispondere alla riduzione di CO_2, il fatto che non siano stati rilevati formiato o alcoli nel HPLC dice che i prodotti devono essere distribuiti in tanti prodotti in concentrazioni troppo basse.

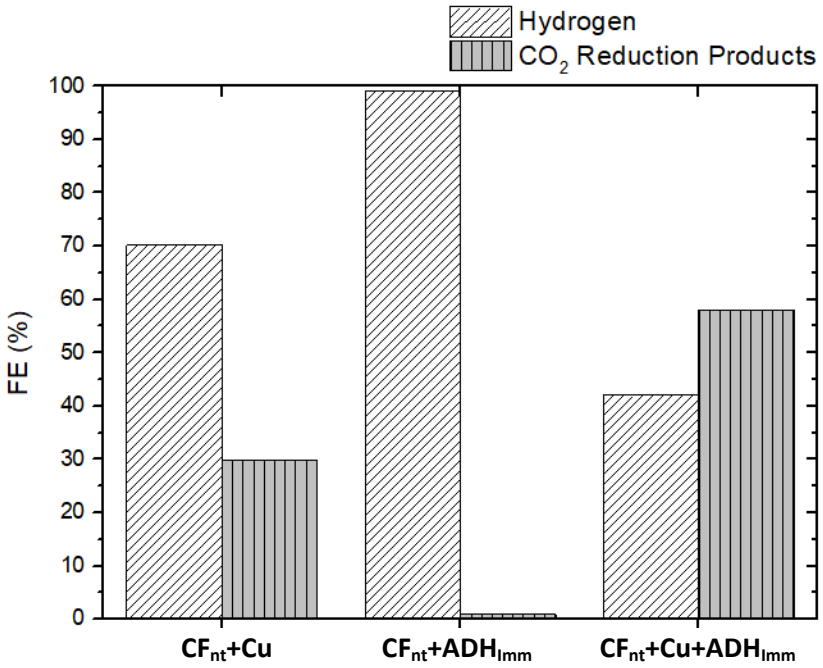

Figura 14. Efficienze faradiche per l'idrogeno e per i prodotti della riduzione della CO_2, per 3 elettrodi: CFnt+Cu, CFt+SnO_2, CFnt+SnO_2 e CFnt+Cu.

Conclusioni

Questo lavoro si centra sulla trasformazione della CO_2 in sostanze chimiche da alto valore aggiunto con un sistema ibrido elettrocatalitico-enzimatico. L'ossido di stagno è stato accoppiato con la FDH per valutare la convenienza di combinare i due tipi di catalizzatori nell'incremento della produzione di formiato. Poi una reazione sequenziale è cercata: la riduzione di CO_2 con rame per ottenere aldeidi, e una seconda fase con l'ADH, per arrivare al metanolo.

L'attività specifica dell'alcool deidrogenasi come enzima libero, nella reazione di ossidazione dell'etanolo a 30°C e pH 7,5, è stata calcolata, 228.99 UI/mg_{enz}. Inoltre, per questo enzima sono stati testati due processi di immobilizzazione. Per l'intrappolamento

con Nafion, non è possibile fare il seguimento del processo e quindi, deve considerarsi che tutto viene fisso sul supporto. Invece, con la tecnica del legame covalente il tempo adeguato di immobilizzazione è stato identificato come 40 minuti e le efficienze di immobilizzazione sono state calcolate dal 32% al 77%. Tra altri il contenuto di aldeidi influenza il rendimento di immobilizzazione; con più aldeidi aggiunti, si hanno delle efficienze maggiore, ma anche viene diminuita l'attività enzimatica a causa di un possibile dispiegamento della struttura 3D dell'enzima.

L'attività immobilizzata è verificata in entrambi tipi di immobilizzazione con valori positivi si concentrazioni di NADH, ma sono stati osservati problemi di ripetibilità. Per l'approccio del legame covalente, non è stato possibile calcolare un valore specifico di attività. L'attività immobilizzata calcolata nel metodo di intrappolamento è stata molto bassa, ma si dovrebbe considerare che c'è anche molta variabilità nel risultato e che una base del calcolo è stata la supposizione di una perfetta immobilizzazione che sovrastima la quantità di enzima fissata. Tuttavia, il metodo di intrappolamento dovrebbe essere più omogeneo ed è anche più compatibile con la deposizione di catalizzatore inorganico, quindi l'intrappolamento con Nafion è stato scelto per fare gli elettrodi per le prove elettrochimiche. La deposizione di ossido di stagno e rame è stata fatta con la tecnica di ricoprimento con spray, con risultati migliori del previsto. Il pretrattamento termico del feltro di carbonio non ha mostrato una differenza rilevante nella quantità di catalizzatore depositato.

Sono stati evidenziati problemi analitici e strumentali per la quantificazione dei prodotti liquidi, principalmente per l'interferenza del cofattore. Per tanto, i risultati dei test elettrochimici sono stati presentati in termini di efficienza dell'idrogeno e, per differenza per completare il 100%, di efficienza faradica di prodotti di riduzione CO_2. Futuri studi possono focalizzarsi sui metodi di quantificazione per migliore lo sviluppo di questi sistemi.

I test di attività elettrochimica nell'elettrodo bianco hanno mostrato correnti elevate, corrispondente principalmente all'HER; ancora 1,6% dell'efficienza faradica è stato trovata per formiato ed acetone. In tutti i CVs dei catalizzatori inorganici si è osservata la preferenza per la riduzione dell'idrogeno e in tutti c'è la produzione di formiato, in maggiore quantità per CF_{nt}+SnO_2, con FE intorno al 7%. Le prestazioni peggiori sono state osservate in CF_t+SnO_2 con efficienze faradica di H_2 più alta (93,6%) e le densità di correnti più bassa. Inoltre, sono stati ipotizzati due problemi riguardanti al trattamento termico: l'aumento del comportamento capacitivo e i problemi di trasferimento di massa della CO_2. Gli sperimenti CF_{nt}+SnO_2 e CF_{nt}+Cu hanno efficienze in prodotti della riduzione di CO_2 molto simili, intorno al 30%, quindi il vantaggio del rame è la quantità di corrente generata.

Di tutti CVs e LSVs gli enzimi hanno le più alte correnti. L'elettrodo CF_{nt}+FDH lascia vedere una preferenza molto chiara per la riduzione di CO_2, invece, per l'ADH entrambe le curve dei CVs sono molto simili, sembra che le proprietà capacitive dell'elettrodo fossero aumentate, questo si rifletta anche sull'elettrodo CF_{nt}+Cu+ADH. La peggiore prestazione si è evidenziata sul CF_{nt}+SnO_2+FDH_{Sln}, se il FDH non reagisce vicino all'elettrodo può essere difficile vedere la reazione, poiché il NADH non si rigenera facilmente. Tuttavia, come anche con la saturazione di azoto, la corrente diminuisce, si suppone che in qualche modo l'enzima deve bloccare l'ossido di stagno.

Un comportamento comune dei sistemi ibridi è l'aumento del corrente rispetto ai catalizzatori inorganici, tuttavia, anche se per CF_{nt}+SnO_2+FDH è stata vista un'efficienza faradica per riduzione di CO_2 inferiore, per il maggiore corrente si otterrebbero maggiori concentrazioni di prodotti che con CF_{nt} + FDH o CF_{nt} + SnO_2. Forze lavorando sul sistema elettrochimico enzimatico l'ibrido può essere migliorato, possibili studi futuri potrebbero essere: l'effetto nella concentrazione di NADH, come vengono distribuiti entrambi catalizzatori e l'effetto dell'aumento del Nafion sull'elettrodo.

Le correnti più elevate si sono visti in CF_{nt}+ADH e CF_{nt}+Cu+ADH. L'ADH non può riconoscere come substrato in nessun modo la CO_2, questo è visto con un'efficienza faradica per il H_2 del 99,13%. Al contrario, l'efficienza faradica di H_2 per CF_{nt}+Cu+ADH è la più bassa, indicando le migliori prestazioni di riduzione della CO_2. Quest'ultimo esperimento ha presentato un profilo di produzione di idrogeno molto interessante, aumentando e diminuendo, a forma di campana, in 150 minuti. Questo potrebbe rappresentare la reazione sequenziale desiderata; prima il Cu produce parallelamente H_2 e prodotti della riduzione della CO2, poi dalle aldeidi prodotte, l'enzima catalizza la riduzione ad alcoli. Tuttavia, non può essere dimostrato senza la quantificazione dei prodotti liquidi e dovrebbe essere ancora spiegato perché l'HER dopo un certo tempo non occorre più.

1. Introduction

1.1. Problem

The global warming is an important worldwide problem; its effects convoy environmental, healthy, economical and politic issues. According to the last report of the intergovernmental Panel on Climate Change (IPCC), the efforts must focus on reducing the increase of average Earth temperature, limiting it to 1.5°C. Different initiatives, as the *Paris agreement* and *Goal 13: Climate action*, are being developed and one of their main concerns around the weather changing is the CO_2 emission reductions. [1]

In 2018 the emissions related to the global energy demand had reached 33.1 Gt, this is an increase of 1.7% respect the previous year and just corresponds to two thirds of the total increase of CO2 emissions [23]. The behavior of atmospheric CO_2 concentrations can be seen in the Figure 1.1; is remarkable that since 2017 the average has exceeded the 400 ppm. [24]

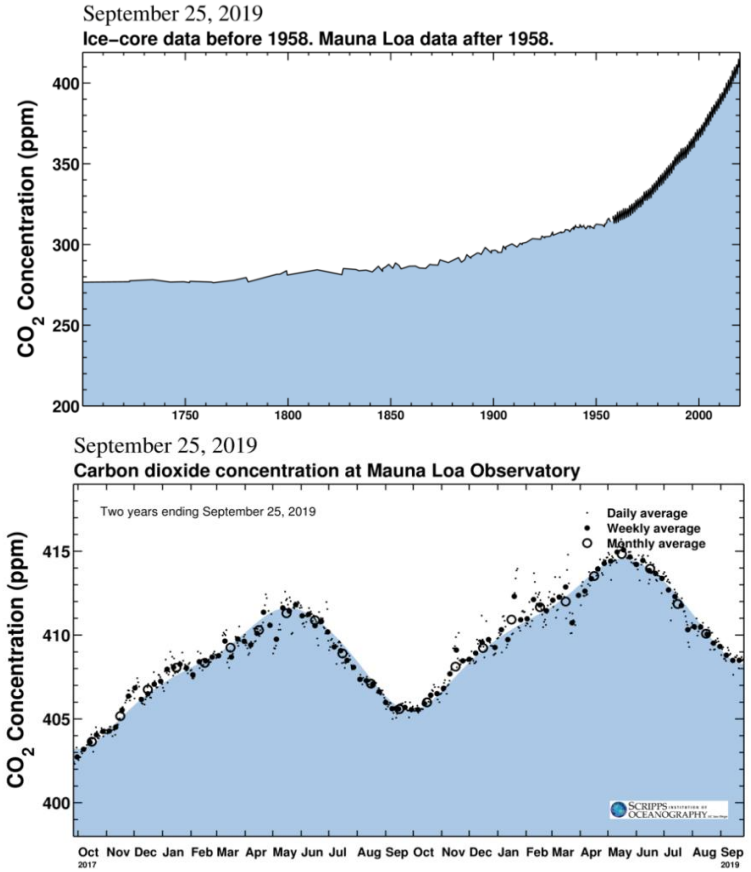

Figure 1.1. Atmospheric concentration behavior of carbon dioxide: (a) annually change from 1700 until 2019; (b) monthly variations from September 2017 until September 2019 with daily, weekly and monthly average values. Reproduced from Ref [24].

The Figure 1.2 summarizes the increase of the emissions from 2017 to 2018; the rise correspondent to the economic growth and how different efforts have helped to counter it are showed. In order from major to smaller contribution, there are reductions because the implementation of energy efficiency policies, the use of renewable energy sources, the transition from coal to gas and the increase of nuclear energy production. It is possible see that the use of this greenhouse gas as raw material for value added products and fuels, has not yet an important effect to appear in comparison with the other. [23]

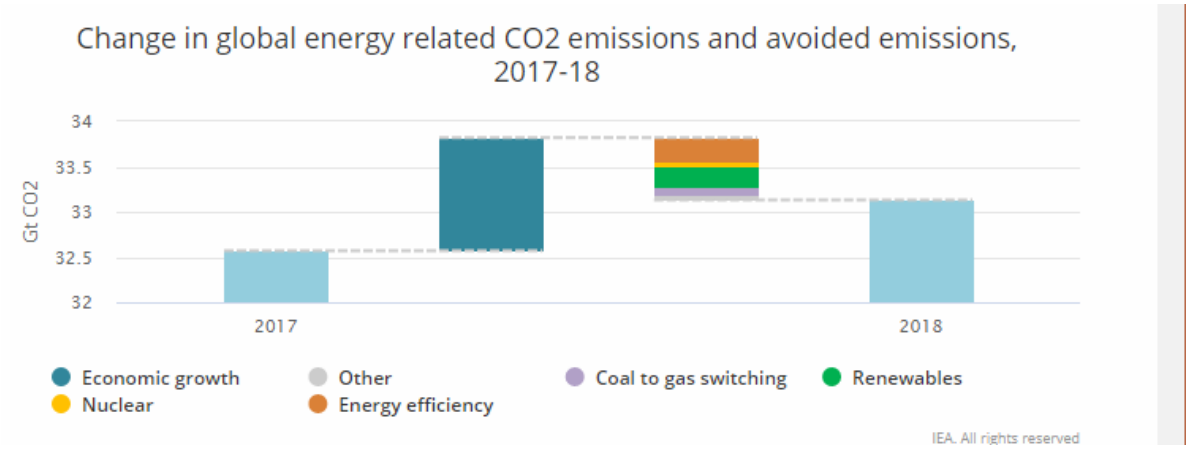

Figure 1.2. Increase of CO_2 emissions from 2017 to 2018 with different kinds of technologies used to reduce it. Reproduced from Ref [23].

In 2016, from the 24 Gt of CO_2 produced with the fossil fuels, the demand was just 200 Mt. It was mainly used for carbonates and urea synthesis, only a very smaller percentage for fuels production as methanol. First basic criteria to know if the production of some compound from CO_2 conversion is economically feasible is to calculate the relative added value, these can be calculated in a rough way with Equation 1.1, where m_p is the mass of product in kg, w_p is the specific value in €/kg, $m_{E,i}$ is the mass of reactant i in kg and $w_{E,i}$ is the specific value of reactant i in €/kg. The data taking different possible costs for CO_2, for different compounds founded in the literature is showed in Figure 1.3. For example methanol leaves in negative added values while formic acid can reach until 200%. To enhance the consumption of CO_2 is necessary work in more efficient and economically feasible fuels production from carbon dioxide reduction. [2]

$$Relative\ added\ value = \frac{(m_p * w_p) - \sum_i^n (m_{E,i} * w_{E,i})}{\sum_i^n (m_{E,i} * w_{E,i})} \quad \text{Equation 1.1}$$

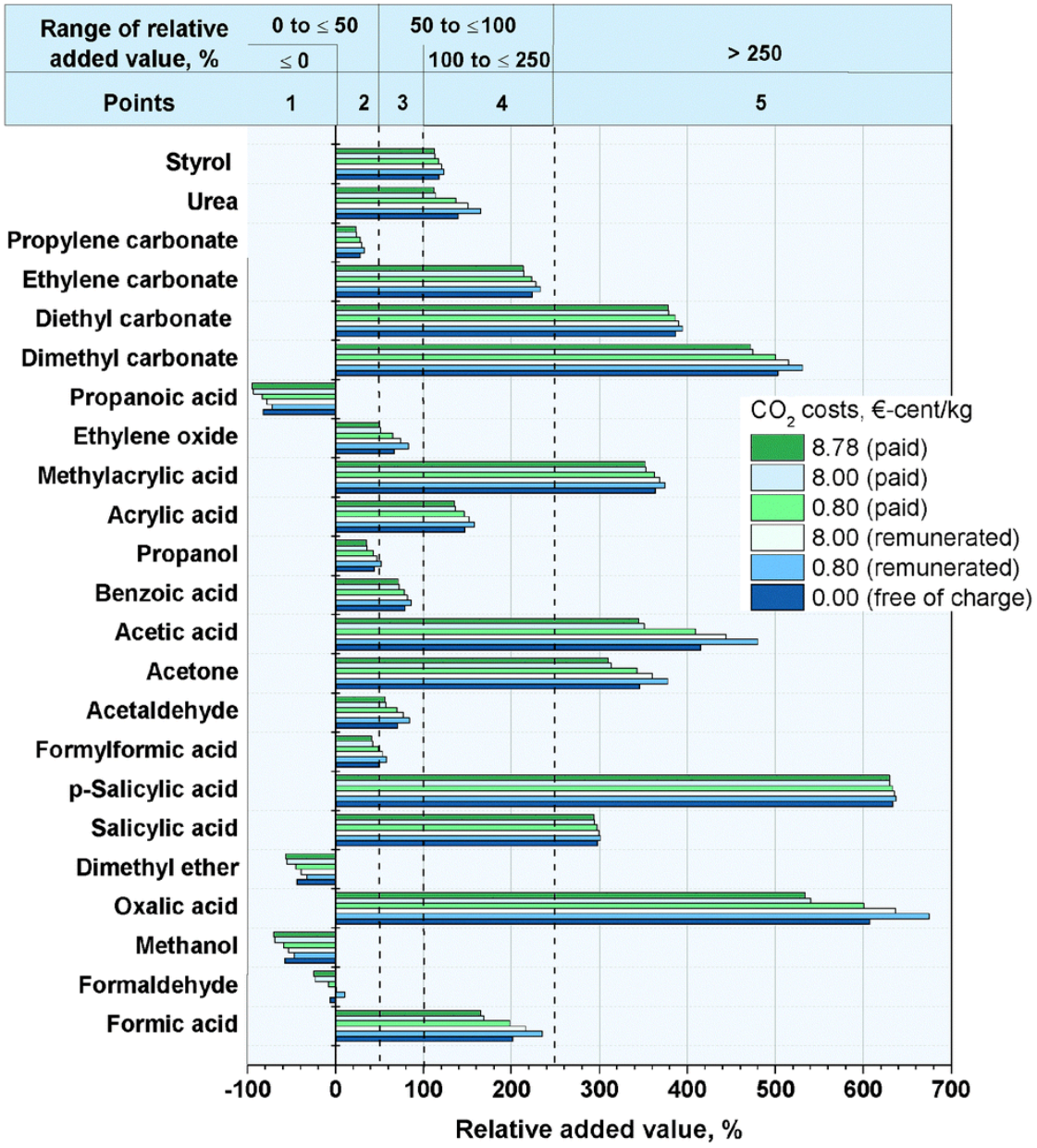

Figure 1.3. Relative added value for different compounds synthesis starting from carbon dioxide. Reproduced from Ref [2].

Approaches for the reduction of atmospheric carbon dioxide concentration are the center of attention in worldwide research. Some are directly focused in CO_2: how to capture it, storage it, its conversion and utilization. Other indirectly methods are working on reducing energetic consumption, finding new energetic sources and how to improve energy efficiencies and production. Speaking about conversion and utilization, there are three different kinds of processes where CO_2 can be used: physical, chemical and biological; the first one is the technological utilization of the gas without its transformation; the other two,

as conversion methods. In the same way the chemical treatments can be divided in different methods: mineralization, electrochemical, thermochemical and photochemical [2]. On the other hand the biological route could be considered as the most ancient technic; also if it was not the main objective, the agriculture and cultivation let to transform the carbon dioxide in biomass, this has evolved with the wide research in genetically modify plants and microalgae, and more recently, the CO_2 biotransformation has been focused in microbial processes and enzymatic approaches. [3]

Carbon dioxide is the principal product in all combustion processes; it is a very stable molecule in both, thermodynamic and kinetic terms. CO_2 conversion to any organic molecule, involving a change in the carbon oxidation state, is a very difficult work and, in general, is endergonic and endothermic processes. The Figure 1.4 shows the free Gibbs energy values of formation for different carbon compounds, the non-spontaneity of the majority of reactions starting from CO_2 can be concluded since the standard reaction Gibbs energies (ΔG^0) are positives, these can be calculated from difference of the standard Gibbs energies of formation (ΔG_f^0) between products and reagents as can be seen in Equation 1.2 [25]. In this way, for all the approaches mentioned previously, the common factor is the necessity of catalyst to reduce the energy required to carry out the transformation. [2]

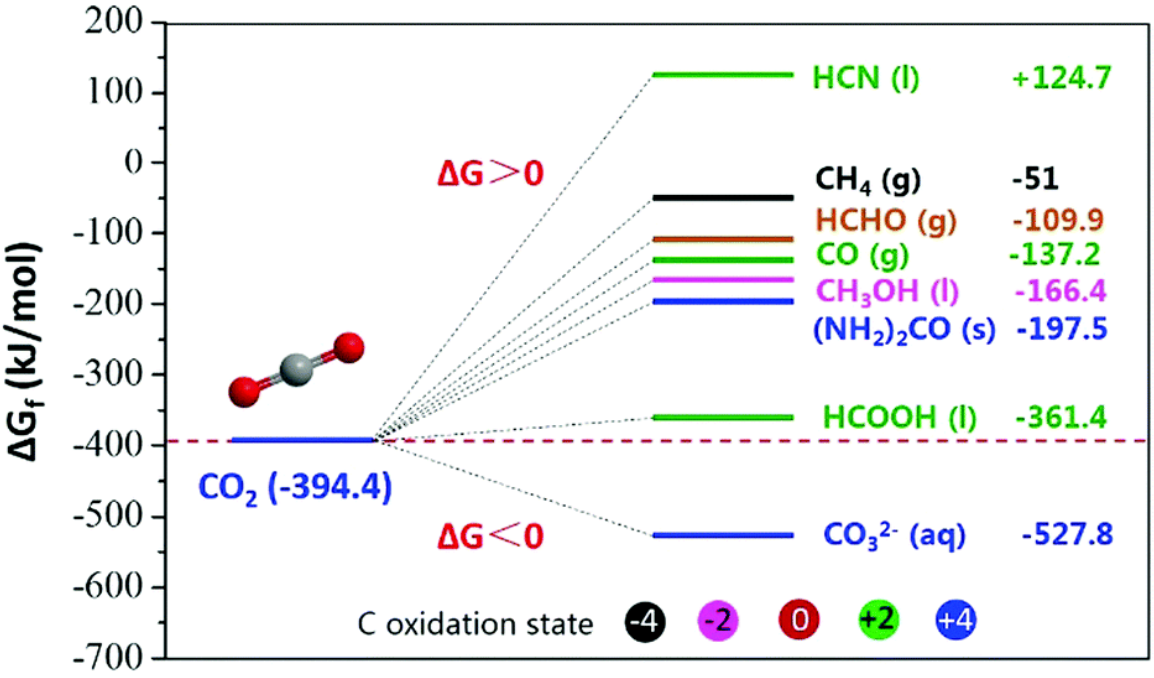

Figure 1.4. Standard free Gibbs energy values for different carbon compounds, the key of colors indicate the oxidation state of the carbon and the sign of the change of Gibbs energy from CO2 to the other molecules is pointed out. Reproduced from Ref [2].

$$\Delta G^0 = \sum_{Products} \Delta G_f^0 - \sum_{Reagents} \Delta G_f^0 \qquad \text{Equation 1.2}$$

Each method named for the CO_2 transformation has some advantages but also a lot of disadvantages that must be improve to be considered a feasible technique and to be used in large scale. Electrochemical reactions, for example, let to achieve transportation fuels, have good controllability, efficiency and scalability but in general have a low selectivity. In contrast the most remarkable benefits of the use of biocatalyst, in addition to being more environmental friendly, are their high selectivity and specificity, but this process are more difficult to control, less flexible with the change of reaction conditions and very instable.

1.2. Scientific background

Important concepts and the state of the art of electrochemical and enzymatic dioxide carbon reduction are presented below.

1.2.1. Enzymatic Reduction

The bio-conversion of carbon dioxide is primarily known for the photosynthetic process in plants, but in general is a fundamental process for life; through complex biosynthetic cycles, solar light and enzymes sequences, CO_2 molecules can be converted in more complex carbon compounds. An example of interest for this work is the production of methanol, where three different enzymes are involved, as can be seen in the Figure 1.5.

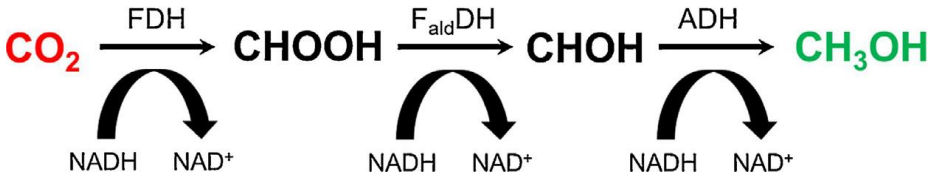

Figure 1.5. Enzymatic cascade in order to trasform carbon dioxide in methanol using the Formate Dehydrogenase (FDH), the Formaldehyde Dehydrogenase (FaldDH) and the Alcohol Dehydrogenase (ADH). Reproduced from Ref [4]

There are three consecutive reactions. First, the CO_2 undergoes a hydrogenation reaction to formate through the enzyme Formate Dehydrogenase (FDH), then, the Formaldehyde Dehydrogenase (FaldDH) catalyzes the reaction from formate to formaldehyde, and finally, formaldehyde is reduced to methanol with the help of Alcohol Dehydrogenase (ADH). Actually, in biological systems, these enzymes do the inverse reactions (i.e. oxidation reaction); this is formate to carbon dioxide (FDH), aldehyde to formate (FaldDH) and alcohol to aldehyde (ADH). An important component of the reaction is the nicotinamide dinucleotide based cofactor (NAD+ in the oxidized form and NADH in the reduced one). In

Figure 1.5, three NADH molecules are oxidizing to NAD+, and in the methanol oxidation direction three NAD+ are reduced. In nature, the NAD+ is regenerated through other enzymatic redox reaction to obtain again NAD+, instead for methanol production the necessity of the cofactor is center of attention as bottleneck of this approach, because a secondary process of regeneration of NADH is required, to avoid the sacrifice of the cofactor in each cycle.

It have been proved that the pathway from carbon dioxide to methanol can be done, but still continues to be inefficient since needed long times of reactions and has very low methanol production. One of the major problems found was the second step, since the FaldDH is highly affected by the substrate – product ratio [5]. Instead alcohol dehydrogenase has showed even preference for the reaction from aldehyde to alcohol [4]. About the FDH, there are two kinds of enzymes, the metal dependent, which has the major reported activities but also with a big disadvantage, it has oxygen labile compounds; and the second type is the NADH dependent, is more stable but there is again the cofactor regeneration problem. Between other factors, also an inhibitory effect of NADH is reported in literature when concentration excess is reached. [16]

Some important definitions are:

- **Enzyme activity:** It is a property to measure how much catalytic work is able to do the enzyme. Even if this is a thermodynamic measure because says how many can the protein reduce the energetic barrier of the reaction, it is easier consider it as a kinetic value, so as the increase in the reaction rate as consequence of the reduction of the energetic requirements. It is totally dependent of the enzyme structure because its capacity to carry out the reaction lies in the active site geometry, which corresponds to a few important amino acids with a very specific configuration to recognize the substrate, make an intermediate conjugate and release the products.

  In a simpler way, the activity is taken as the maximum reaction velocity (Vm). The reaction rate decreases with time until reaches a zero value and increases with the substrate concentration until arrives to a constant tendency. To obtain the maximum rate possible, the activity is taken as the initial velocity and should be calculated at substrate concentrations over 5 times the Michaelis constant because, in general, can be considered that at this point the stationary velocity is reached (Km: Substrate concentration such that V=Vm/2). [6]

  From here the specific activity is defined as shows the Equation 1.3, where one international Unit (IU) refers to the quantity of enzyme necessary to convert one micromole of substrate or product in one minute at determines set of conditions. [9] For ADH and FDH, the reported specific activity was measured at pH 7 and 30 ºC.

$$\text{Specific } Enzyme\ activity = \frac{UI}{mg\ enzyme} = \frac{\mu mol\ NADH/min}{mg\ enzyme} \quad \text{Equation 1.3}$$

- **Immobilization:** The protein immobilization is a fundamental part in the development of enzyme systems with industrial application; it is a process for fixing the protein in solid supports. Some of the major disadvantages of biological approaches, as cost, stability, isolation and purification are improved through the use of appropriate material and efficient immobilization methods. In addition, immobilization permits to reuse the biocatalyst.

  These methods can be physical or chemical; on this work were evaluated two different immobilizations, covalent bonding and entrapment. The first one uses the amino groups of the lysine residues of the enzyme and the aldehyde groups created on the support with a previous functionalization, respectively the red and blue initial reagents shown in the Figure 1.6. At suitable pH the nitrogen is deprotonated and a nucleophilic addition happens, it is followed by dehydration and leaves a Schiff bond that can be reduced with sodium borohydride in a covalent bond. The second one, simpler method, uses a polymeric cover, it should be inert for the protein and must let pass substrates and products while the enzyme is retained. In this approach an immobilization solution composed by a solvent, the polymer and the enzyme is apply on the support, when the solvent is gone (by drying in desiccator to avoid the protein denaturalization) an arrangement as that one showed in Figure 1.7 is expected.[26]

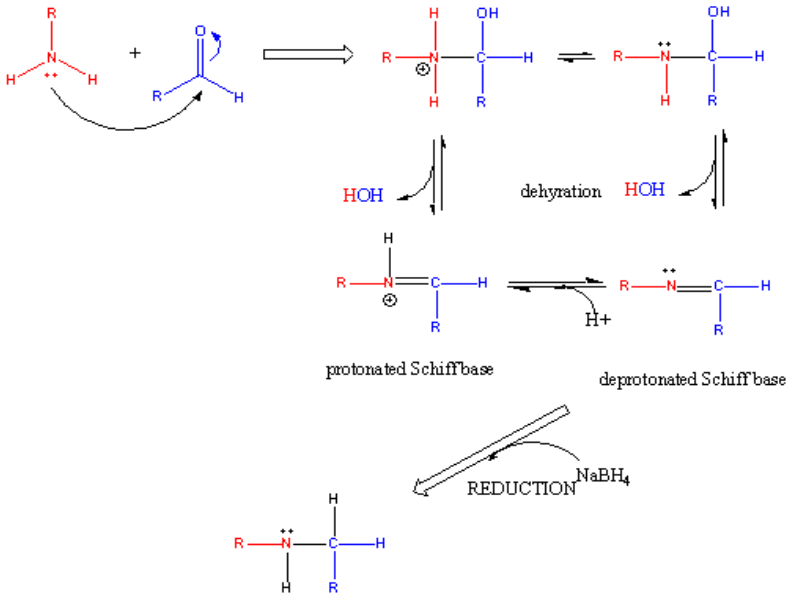

Figure 1.6. Reaction mechanism of covalent bond formation for enzyme immobilization on pre functionalized material. Reproduced from Ref [27]

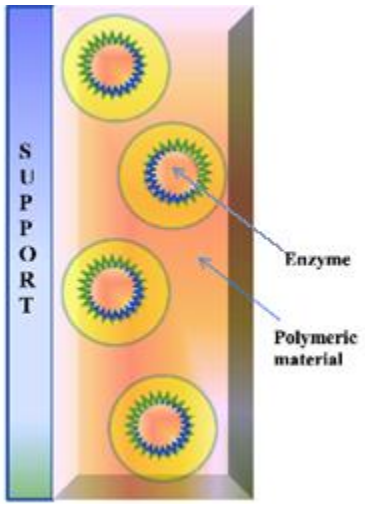

Figure 1.7. Entrapment immobilization technic. Reproduced from Ref [26]

Even if the protein stability comes improved with the increase in the enzyme rigidity, the activity usually is also affected with the immobilization, since the bond with the support occurs in a random way, so the active site can be blocked, deactivated, denaturalized, etc. The immobilization also can have a cost in mass transfer terms depending of the material porosity. It is necessary to define two different parameters to evaluate the suitability of the immobilization method.[7][8]

- **Immobilization efficiency:** It is the estimation of the quantity of enzyme supported and is expressed in percent value respect the initial enzyme quantity offered to the material support. [9]

- **Retained activity:** Also considered as the immobilization yield is the relation between the activity measure in the immobilized enzyme and the equivalence in free enzyme activity of the enzyme quantity given at the support. [9]

1.2.2. Electrochemical Reduction

The theory basis of the electrochemical reaction is the coupling of two different reactions in two different electrodes, one of reduction and other of oxidation, generating an electron flow between them. The direction of the spontaneous reaction depends on the potential; the electrons go from the lowest to the highest one. For generating the opposite current, it is necessary to add an external work using an external voltage source; mathematically this can be seen with the sign of the total cell potential, positive when the cell provides energy and negative when energy must be supply to the circuit. The potential of the complete cell is given by the difference of the potential of the cathode, where the reduction occurs, and the potential of the anode, the oxidation side. [10]

The electrochemical conversion of CO_2 to small carbon compounds, as methanol, is realized at low temperatures, some reactions with their equilibrium potentials are shown in Table 1.1, using as reference a reversible hydrogen electrode. It is possible see that with the increase of electrons the potential goes to more positive values, in thermodynamically terms, when more electrons are involved in the reaction, the required free energy is lower. [2]

Table 1.1. Electrochemical semi-reactions of CO_2 reduction with the number of electrons involved and standard potentials calculated with reversible hydrogen electrode. Reproduced from Ref [2]

Electrode reaction	E^* (V) vs. RHE (V)
$CO_2 + e^- \rightarrow CO_2^-$	−2.14
$CO_2 + 2e^- + 2H^+ \rightarrow HCOOH$	−0.85
$CO_2 + 2e^- + 2H^+ \rightarrow CO + H_2O$	−0.76
$CO_2 + 4e^- + 4H^+ \rightarrow HCHO + H_2O$	−0.72
$CO_2 + 6e^- + 6H^+ \rightarrow CH_3OH + H_2O$	−0.62
$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O$	−0.48

As mentioned first above, the reduction of CO_2 is a very difficult process, in electrochemical ways, it needs a high overpotential, mainly because the reduction of CO_2 molecule to the intermediate CO_2^- has a potential of -1.9 V (vs RHE at pH 7) [11]. However, this energy requirement can be reduced with the use of electrocatalyst. Some important parameters to follow the electrochemical reactions are the faradic and energetic efficiencies; there is a clear relation between both, high Faraday efficiency and low overpotentials are looked at to obtain better values of energetic efficiencies. [2]

- Faradic efficiency: It is expressed as the ratio between the energy spent to produce one mole of desired product and the total energy passing through the system. It gives a measure of the selectivity. In the mathematical expression showed, Equation 1.4, z is the number of electrons, F is the Faraday constant, n is the number of produced moles and Q is the total current.[2]

$$\varepsilon_f = \frac{zFn}{Q} \qquad \text{Equation 1.4}$$

Different metals and oxides have been studied in the electrochemical reduction of CO_2; the Figure 1.8 summarizes the most common metals used. The acid formic production with metal surfaces as Sn [12], [13], In [2], Pb [2] and Hg [2], and the carbon monoxide synthesis which use for example Au [2] or Ag [14] are remarkable processes because its high selectivity. Instead the cupper catalysts are interesting since a lot of different compounds can be obtained [14], [15], a compilation of the mechanisms are presented in Figure 1.9. In this case the pathway reactions are being studying to improve the selectivity

[28], [29]; get a better understanding of the active sites and the reaction mechanism could led to a more controllable process [30]. Other researches have been working to prove the vantages of oxides and nanomaterials as catalysts, the results indicate that they can improve the catalytic activity, the selectivity and the Faradic efficiency. [2]

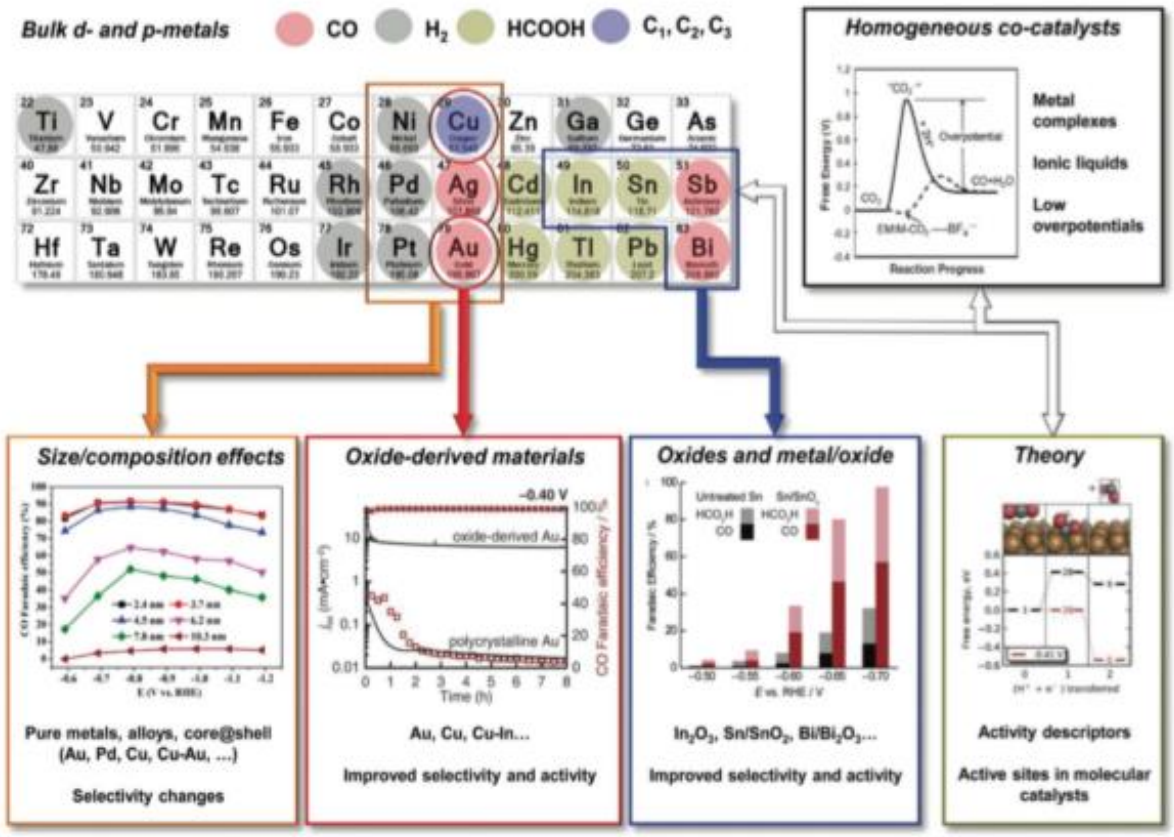

Figure 1.8. Main elements used as electrochemical catalyst, in both, bulk and oxides form. There is also indicated the principal products and some characteristics about their use. Reproduced from Ref [2].

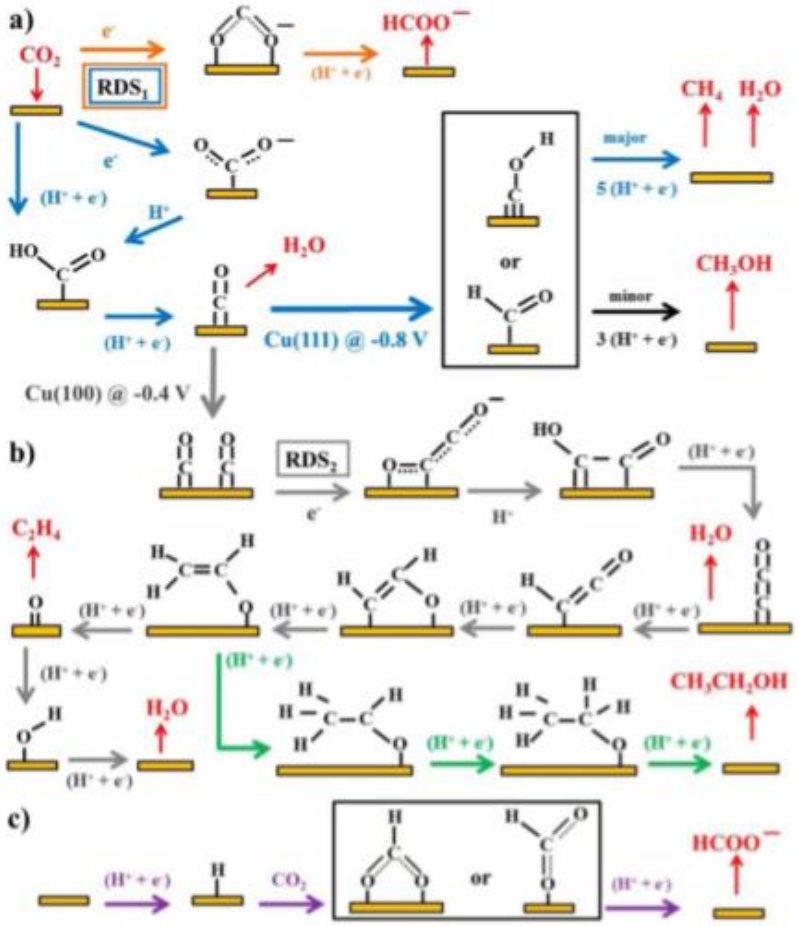

Figure 1.9. The proposal reactions mechanisms on cupper catalysts are presented. The pathways to (a) formate, methane, methanol, (b) ethane, ethanol and (c) formate are showed. Reproduced from Ref [2].

1.2.3. Bio-electro catalytic hybrid system

Some goals have been attempted in the implementation and development of CO_2 conversion process, but there is still a lot of work to do at technical, as well as social levels, including energy requirements, technology development, market size and investments. Specifically, about chemical and engineering areas concern, research must be still made to have more understanding in the reaction mechanisms, in the increment of the selectivity, the efficiencies and the stability of known and new catalysts. In the same way a new investigation line is the combination of two different kinds of catalysts, these ones are enzymes and electrocatalysts, to obtain a more complete arrangement. Some examples are mentioned bellow.

Some approaches involve the whole microorganisms, they can take directly the CO_2 through the enzymes in its interior and use it in their electron transference processes; others

attempts use the isolated enzymes, in this way, the purification processes are easier because reduce the co-products, also let to choose the better enzymes, of the better microorganisms to optimize the multi enzymatic reactions. About the first case, the utilization of complete microorganisms, there are three types of systems and can be seen in the Figure 1.10: the microbial full cell (MFC) where the microorganism is put in the anode to carry out the oxidation reaction while in the cathode the reduction of oxygen takes place; the microbial electrolysis cell (MEC), as in previous cell the biological agent is put in the anode, but this time, the cathode reaction has a lower voltage and external potential must be apply; the last one and that one of major interest is to pass from CO_2 to complex molecules as fuels is the microbial electrosynthesis, it is carry out through a bioreaction reduction in the cathode. [10]

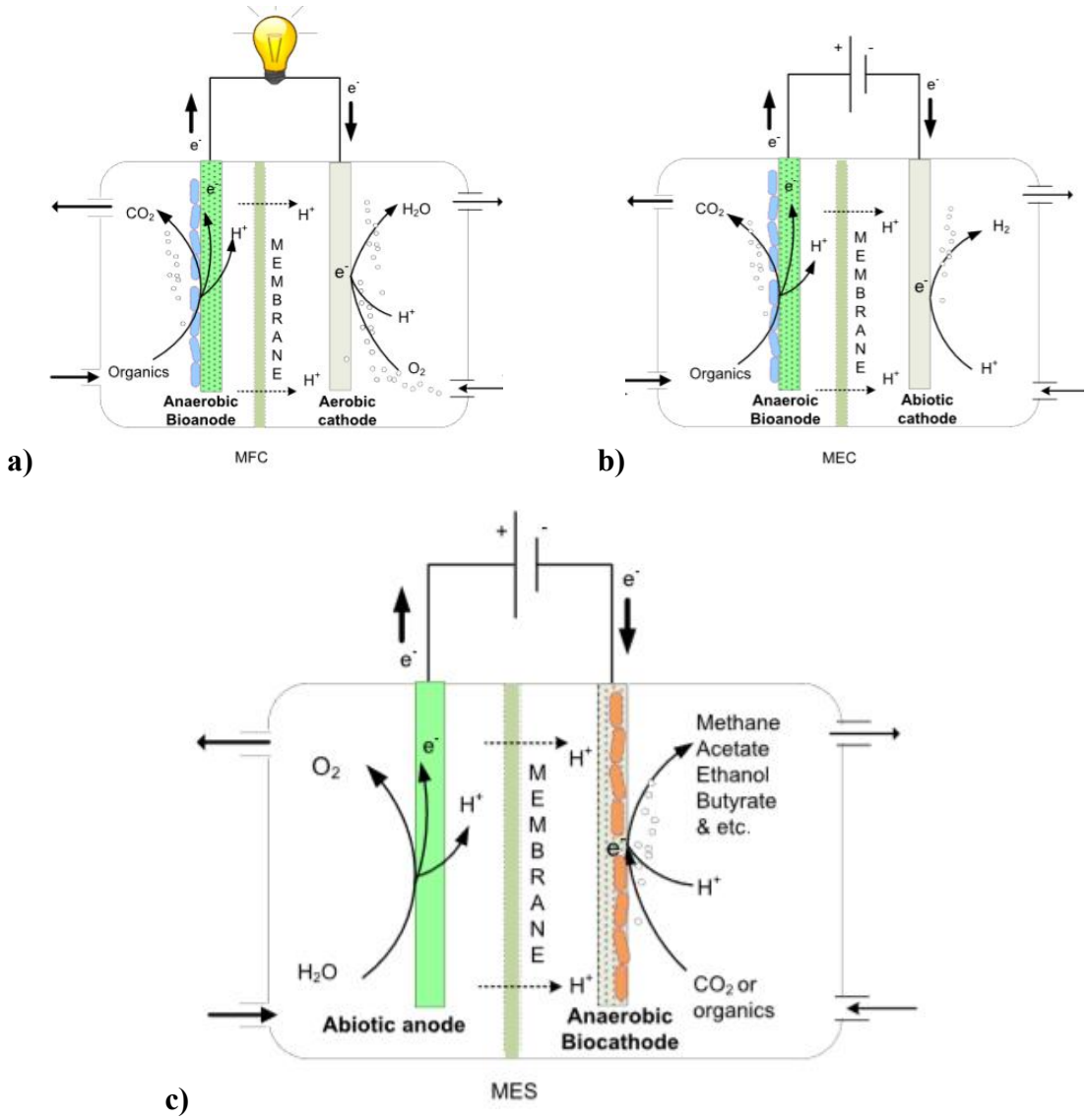

Figure 1.10. Bio–electrocatalytic systems with whole microorganisms are indicated. (a) Microbial full cell (MFC), (b) microbial electrolysis cell (MES) and (C) microbial electrosynthesis. Reproduced from Ref [10].

Other example of hybrid systems is the use of the enzyme cascade previously named (FDH, FaldDH, ADH) with a photo-electrocatalytic system, Co-Pi/α-Fe_2O_3 // $BiFeO_3$, [13]. With the cascade the CO_2 becomes methanol and the photo-reaction is carried out to get the NADH regeneration. This experimentation set is closer to the ensemble realized to this work in the sense that enzymes are used to one part of the reaction and the electrocatalytic system to other. [11]

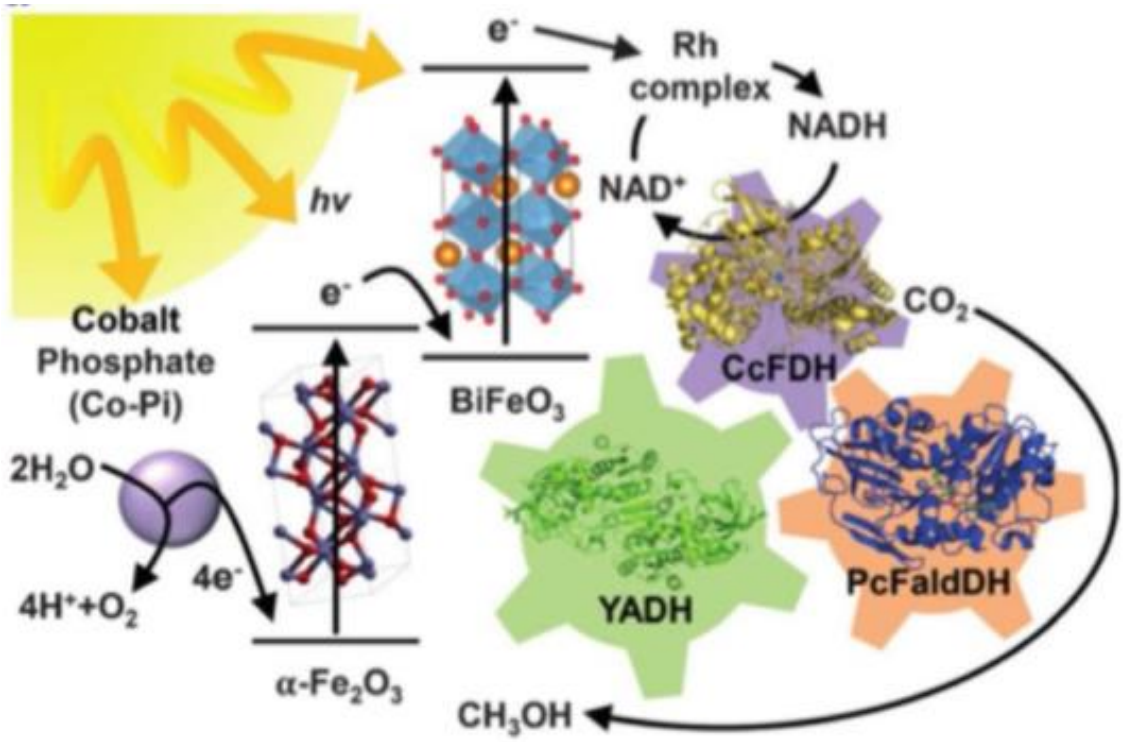

Figure 1.11. Sequential enzymatic reactions composed of Formate Dehydrogenase (FDH), Formaldehyde Dehydrogenase (FaldDH) and Alcohol Dehydrogenase (ADH) for CO_2 reduction to obtain methanol, with photo-electro catalytic system formed by Cobalt Phosphate (Co-pi), α – iron oxide (α-Fe_2O_3) and Bismuth ferrite ($BiFeO_3$) acting on Rhodium complex to regenerate NADH cofactor. Reproduced from Ref [11] .

A similar mechanism, with electrochemical catalyst instead of photocatalytic approach is showed in Figure 1.12. In this case, the enzymatic reaction is just to reduce the carbon dioxide in formate with the FDH, while the NADH regeneration is reached with the oxidation of a rhodium complex, which must be also regenerated, using an electrochemical cell composed by a copper cathode where the Rh (I) go to Rh (III) complex and platinum anode where the oxidation of water is done. A remarkable result of this experiment is that the regeneration system seems to produce also formate. [16]

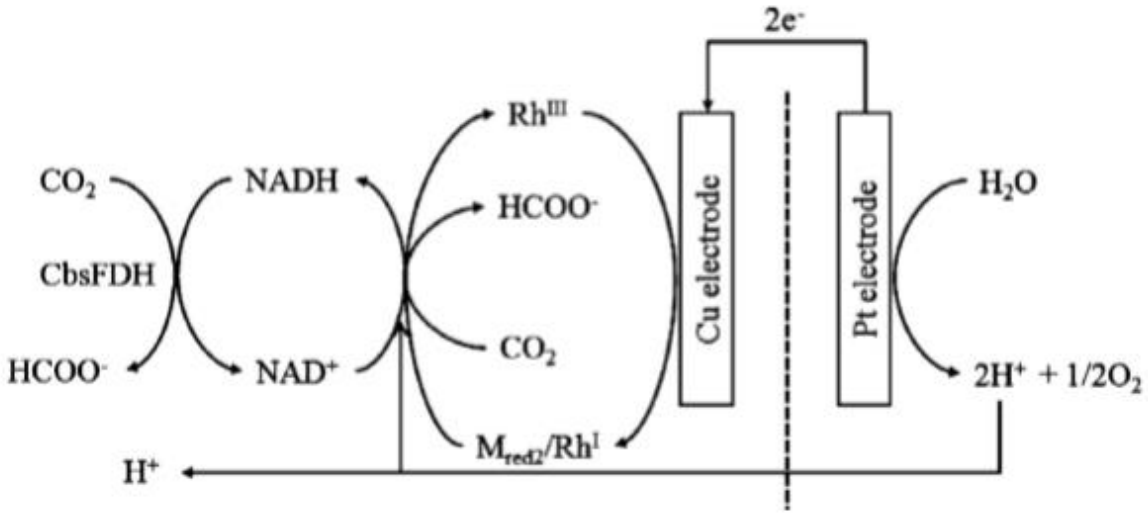

Figure 1.12. Formate synthesis from CO_2 reduction using a Formate Dehydrogenase from *Candida Boidinii* (CbsFDH) enzyme with an electrochemical system used to the NAD+ reduction. The NADH regeneration is carried out with a rhodium complex, in turn, regenerated on cupper with an electrochemical reaction. Reproduced from Ref [16].

1.2.4. Support material

The dehydrogenase enzymes are redox proteins that use the NADH cofactor for the electron transference, so the conductive properties of the material are of great interest. To choose the material there is still fundamental the mass transport behavior, to avoid interferences with the substrate consume and to aid for the easy release of products. Previous studies have proved immobilization in materials as hybrids alginate-silicate matrix and gels, also immobilization with no covalent binding in polymeric membranes, however there is few information about the use of felt supports, one of the closest example is the crosslinking immobilization of fructose dehydrogenase in carbon felt with glutaraldeyde. [31][17]

In general carbon materials have been very used as electrodes. About the different carbon based options, the carbon felt has an advantage compromise between its price, the porosity, the surface area and the conductive properties; this means a cheapest material with high quantity of active sites, good mechanical resistance and excellent electric conduction; furthermore, the carbon felt can be functionalized with specific groups that make it possible the bond support-enzyme. In contrast, has some disadvantages as the low wettability and consequently, because its hydrophobicity, low activity in aqueous solution. [17]

Looking for the material performance improves a long list of treatments has been explored, for example chemical, thermal and plasma treatments; also metallic, graphene, carbon nanotubes nanofiber, polymer and zeolite based modifications. The thermal and chemical

treatments are some of the most commonly found in literature and also the simplest to do, many times they are used in combination and both aid to the enhance of wettability, number of active sites and conductivity, resulting in a higher catalytic activity; both methods work through the increment of the carbon-oxygen groups concentrations. [17]

1.2.5. Objective

This work focusses on the transformation of carbon dioxide in value added chemicals, such as fuels, with a combination of chemical and biological technics, an electrocatalytic-enzyme hybrid system. In this way, two main arrangements are tested.

The research done by Bejtka et al [13] found suitable results about the use of tin oxide in acid formic production, so as first experiment the same electrocatalyst is coupled with the formate dehydrogenase enzyme, that also is able in reduce carbon dioxide in formate. To evaluate the desirability to combine both kind of catalysts the CO_2 reduction with each one is compared with the production of the coupled system.

After, a complete pathway to arrive from carbon dioxide to methanol is searched, so sequential reaction is tried. The CO_2 reduction is carried out with cupper based catalysts, which previously have presented aldehydes production, and the second reaction step is made with the alcohol dehydrogenase, able to synthetize alcohols from aldehydes.

In both cases the research looks for the electrocatalytic deposition, through spray coated technique, and the protein immobilization, by the entrapment method; both in the same piece of carbon felt.

2. Experimental Methods

2.1. Biocatalytic system

2.1.1. Activity measure of free enzyme

The activity of the enzymes is calculated in terms of the oxidation reactions, the conversion of ethanol to acetaldehyde for the alcohol dehydrogenase (ADH). The reaction is showed in Figure 2.1, the molecule used to follow the reactions is the NADH and, since it absorbs at 340 nm, spectrophotometer technic is employed. The reaction takes place directly in the spectrophotometer cuvette; first the substrate is carried (40°C), then the cofactor NAD+ is added (room temperature) and finally the enzyme solution is aggregated (4°C); all solutions are done with buffer phosphate 100 mM at pH 7.5 (KH_2PO_4/K_2HPO_4, Sigma Aldrich, 99%/98%). The reaction begins with the enzyme addition, so at that point the cuvette is located in the spectrophotometer (BioMate 6, Thermo Scientific); the temperature inside the cuvette is 30°C. The Table 2.1 summarized the reagents used.

A)

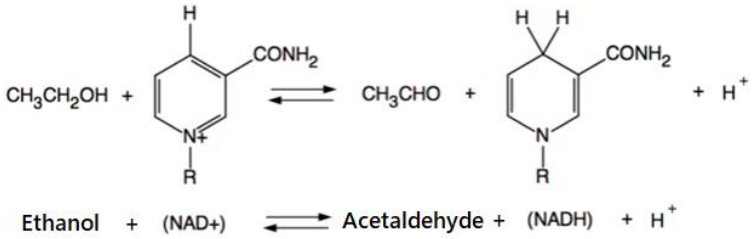

Figure 2.1. Reaction used to measure the enzyme activity: Ethanol oxidation to acetaldehyde by Alcohol Dehydrogenase with NAD+ as cofactor.

Table 2.1. Reagents used to enzymatic activity measure with concentrations and quantities for ADH enzyme.

Enzyme	Solution	Reagent	Concentration	Quantity	Specifications
ADH	Substrate	Ethanol	250 mM	1 ml	S. Aldrich, 99.8%
	Cofactor	NAD+	100 mM	0.050 ml	GERBU, >93%
	Enzyme	ADH	0.01, 0.005 mg/ml	0.025 ml	Worthington, 100%

The increase of the absorbance due to NADH formation is measured for 1 minute. The enzyme activity of soluble ADH in UI/mg is calculated through the Equation 2.1, where $\Delta A/\Delta t$ is the slope of the change of absorbance in function of time, ε is the absorptivity constant previously determined with a calibration curve of absorbance in function of NADH concentration as 4.77 µmol/abs*ml, V_{tot} is the reaction volume, V_{enz} is the enzyme solution volume and F_{dil} is the dilution factor, with value 100 for 0.01 mg/ml concentration and 200 for 0.005 mg/ml concentration.

$$Enzyme\ activity = \frac{\Delta A}{\Delta t} \cdot \frac{1}{\varepsilon} \cdot \frac{V_{tot}}{V_{enz}} \cdot F_{dil} \qquad \text{Equation 2.1}$$

2.1.2. Covalent bond immobilization

- Carbon Felt (CF) functionalization

The immobilization onto functionalized CF was performed only for the ADH enzyme. Firstly, covalent bonds between the material and the 3-Glycidyloxypropyl-trimethoxysilane (GPTMS) are generated, for this the support is put in a round-bottomed flask with toluene (60 ml/gCF) and the GPTMS (3.7 and 0.5 % v/v were proved). The reaction is carried out at 105°C by 5 hours with agitation and reflux condensation; at the end of the process the material is rinsed and filtrated with acetone and water. In the second step a hydrolysis is made with the same previous experimental set at 85°C, sulfuric acid (0.1 M, 60 ml/gCF) is aggregated, in this way, the epoxy rings are opened and hydroxyl groups are formed [18]; again the material must be rinsed and filtrated. Finally, the hydroxyl are oxidized in glyoxal groups [19], it is made through an oxidation with sodium periodate (0.1 M, 60 ml/gCF), this last reaction is done at room temperature by 2 hours in agitation. Samples must be taken from the initial solution of periodate and from the supernatant at the end of the oxidation to quantify the aldehydes added to the carbon felt. The final support must be washed with water and phosphate buffer solution of 7.5 pH.

- Aldehyde groups measurements

The surnatant at the beginning and at the end of the reaction with periodate solution are analyzed by spectrophotometer; 0.1 ml of the sample is mixed in a cuvette with 0.5 ml of sodium carbonate (Saturated solution) and 0.5 ml of potassium iodide (10% w/w). The cuvette is placed in the equipment and the absorbance at 420 nm is measured. With the results the number of aldehyde groups on the surface (N_{Ald}) can be calculated using the Equation 2.2, where $V_{[IO4-]}$ is the volume in milliliters of sodium periodate, the concentration of iodate ions, $C_{[IO4-]}$ is 0.1 M, Abs_f and Abs_i are respectively the spectrophotometer results of the final and initial sample, and at least all is divided by the quantity of support functionalized.

$$N_{Ald} = \frac{V_{[IO_4^-]} x \left(C_{[IO_4^-]} - C_{[IO_4^-]} x \frac{Abs_f}{Abs_i} \right)}{g\ CF} \qquad \text{Equation 2.2}$$

- Immobilization

The immobilization procedure can be done in two stages, the called Shiff bond formation and its conversion into a covalent bond. The first one is strongly dependent of the pH; the enzyme needs a basic medium, over 10, to use the lysine residues to react with the aldehyde groups of the support. According to this, 1 g of the material CF is put in agitation, at 4°C, with 80 ml carbonate buffer pH 10.05 (0.1 M) and the enzyme. Different quantities of protein were tested (0.5, 1, 4 mg ADH/gCF). To monitor the immobilization, samples of 1 ml of the supernatant are taken each 20 minutes, these ones are used to measure the activity of the free enzyme and the remainder quantity of protein; the immobilization time can be decided when the quantity of protein in the supernatant and its activity tends to be constant. When that point is reached, sodium borohydride is added (0.5 mg/ml) to achieve covalent bond. The reaction continues for 10 minutes more, after this the material is rinsed with water, then with buffer pH 7.5 and dried in desiccator.

- Protein content

The mass of enzyme is measured following a standard procedure. In a spectrophotometer cuvette 1 ml of Bradford reagent is mixed with 0.1 ml of the sample, both reagents should be gently swirled and is necessary wait 15 minutes first to take the cuvette for the equipment. The absorbance obtained at 596 nm can be converted in protein quantity with a suitable calibration curve, Figure 2.2.

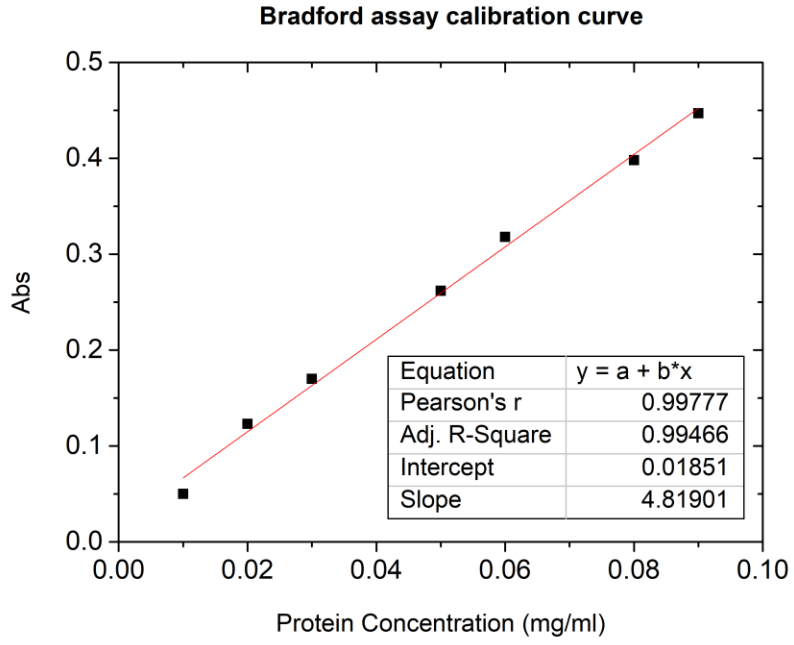

Figure 2.2. Calibration curve for protein concentration in function of the absorbance for the Bradford technic.

In the Table 2.2 the specifications of all the reagents used for the different procedures of the covalent bond immobilization are summarized.

Table 2.2. Specifications of the reagents used in the covalent bond immobilization

Reagent	Specifications
Toluene	S. Aldrich 99.8%
GPTMS	S. Aldrich > 98%
Sulfuric acid	S. Aldrich, 95-97%
Sodium periodate	S. Aldrich > 98%
Sodium carbonate	S. Aldrich >99%
Potassium iodide	Alfa Aesar 99%
Sodium Bicarbonate	S. Aldrich >99%
Sodium Borohydride	S. Aldrich >96%
Bradford reagent	S. Aldrich

2.1.3. Entrapment Immobilization

An immobilization solution is prepared and added to the support with a micropipette. The quantities of the reagents used for 1 g of carbon felt are described in Table 2.3. The solutions should be applied homogenously in both support surfaces and then must be taken to desiccator until the material becomes dry.

Table 2.3. Reagents and proportions to prepare an immobilization solution for the entrapment technic.

Reagent	Quantity	Specifications
Nafion	3.5 ml	Aldrich, 5%
Isopropanol	3.5 ml	Aldrich, 99.8%
Phosphate buffer 7.5 pH 0.1 M	1 ml	KH_2PO_4/K_2HPO_4 – Aldrich, 99%/98%
Enzyme (solution in buffer)	4 mg (1 ml)	Worthington, 100%
Total Solution	9 ml	-

2.1.4. Measurement of the Activity of the immobilized enzyme

Measures of the enzyme activity are done for the ADH enzyme immobilized in the carbon felt, for both covalent bond and entrapment technics. Two different procedures were tested:

- Approximately 10 mg of immobilized carbon felt are put in 2 ml of ethanol solution (250 mM, in buffer phosphate 0.1 M at pH 7.5) at 30°C, the recipient is placed in a heated plate to maintain the temperature and slow stirring is used. When the carbon felt fibers look dispersed in the solution, 0.050 ml of phosphate buffer of 7.5 pH are

aggregated and finally 0.1 ml of NAD+ are added, at this point, with the cofactor in solution, the reaction begins. The production of NADH in the surnatant is measured spectrophotometrically (at 340 nm) at different reaction times. To avoid any interference with the analysis, the cuvette should be free of carbon felt fibers . To calculate the activity is necessary to do at least 3 tests with different reaction times, in this way the absorbance can be plotted in function of time and the slope can be used in the Equation 2.3.

- Bigger pieces of carbon felt were also tested (approximately 0.1 g), whereas the proportion of all reagents are conserved. In this way 20 ml of ethanol solution (250 mM in phosphate buffer 0.1M pH. 7.5) are used, 0.5 ml of buffer solution are added and, at least, 1 ml of cofactor to begin the reaction. In this case different samples are taken from the same experiment at certain times, for example at 1, 2 and 3 minutes; these ones are evaluated by spectrophotometer and the absorbance is plotted in function of time to calculate the slope and use the Equation 2.3.

$$Enzyme\ activity = \frac{\Delta A}{\Delta t} \cdot \frac{1}{\varepsilon} \cdot \frac{V_{tot}}{mg_{enz}} \qquad \text{Equation 2.3}$$

2.1.5. Evaluation of the immobilization process

Two parameters are determined to evaluate the suitability of the immobilization process, the immobilization efficiency and the percentage of retained activity. The first calculation can be done from the Equation 2.4, on the basis of the protein mass change, calculated from the supernatant with the Bradford method; C correspond to the concentrations found, V to the remaining volume in the reaction and the index i and f indicate, respectively, the initial and final time of reaction .

$$IE = \frac{C_i V_i - C_f V_f}{C_i V_i} \qquad \text{Equation 2.4}$$

The retained activity is the comparison between the activity as free enzyme of the quantity of immobilized enzyme and the activity showed on the support, Equation 2.5.

$$\%Activity = \frac{Immobilized\ enzyme\ activity}{Free\ enzyme\ activity} \qquad \text{Equation 2.5}$$

2.2. Electrochemical catalysis

2.2.1. Electrochemical catalyst deposition

- Support thermal treatment

In some experiments the carbon felt is subjected to a calcination to improve its wettability properties. The material is placed in a furnace and a thermal ramp is programmed with a heating of 10°C by minute until 500°C, where temperature is maintained by 5 hours.

- Ink elaboration

The reagents quantities for the ink are calculated proportionally to the geometrical surface area of the electrode, as detailed in Table 1.4. First, the catalyst and binder are added and sonicated until a homogenous dispersion is obtained, then the solvent is added and the mixture sonicated again. Table 2.4

Table 2.4. Reagents, proportions and specification for the ink

Item	**Ink 1**		**Ink 2**	
Catalyst	SnO_2	Aldrich, <100 nm	Cu	Aldrich, 40-60 nm
Expected catalyst Load (mg/cm^2)	3	-	3	-
Efficiency expected (%)	30	-	30	-
Binder	Nafion	Aldrich, 5%	Nafion	S. Aldrich, 5%
Catalyst/Binder ratio (w/w)	72/28	-	72/28	-
Solvent	Isopropanol	Aldrich, 99.8%	Isopropanol	S. Aldrich, 99.8%
Solid (%w/w)	5	-	5	-

- Spray coated deposition

All the depositions are made with a spray gun using nitrogen gas as carrier. The carbon felt is covered with a mask of an inert material, which let exposed the correct shape and size of the electrode. This arrangement is placed on a hot plate at 100°C for 20 minutes to remove the humidity. Once the support is dry, it is possible to proceed with the spray covering, which should be done in the more homogenous possible way and trying to reduce the losses of the ink at the outside of the area desired. The deposition is performed on the hot plate at 100 ° C and the temperature is maintained until the complete evaporation of the solvent. An example of the experimental set and the spray gun used are presented in the Figure 2.3.

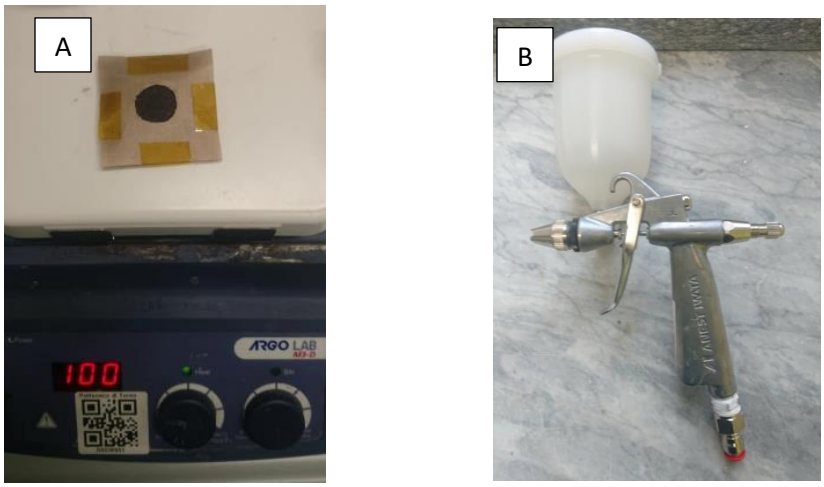

Figure 2.3. Experimental set used to spray coated deposition. A) Arrangement of carbon felt with an inert mask in hot plate. B) Spray gun used to deposition.

The carbon felt is weighted before (CF1) and after (CF3) the deposition step to calculate the quantity of deposited catalyst. The catalyst load (in mg/cm^2) is calculated as follow, where A is the area of the electrode (3.14 cm^2) and Rcat is the fraction of catalyst in reference to the total of solids (0.72).

$$Catalyst\ load = \frac{CF2 - CF1}{A} * R_{Cat} \qquad \text{Equation 2.6}$$

2.2.2. Electrocatalytic activity

2.2.2.1. Experimental set

- Electrochemical cell

The electrochemical cell has 2 chambers, the cathodic one (with a volume of 20 ml) , and the anodic one (with an operative volume of 40 ml). A polymeric membrane (CMI 7000 cation exchange membrane) is placed between both sides and, to guarantee the sealed, two gaskets are put at each chamber; the membrane does not allow the transfer of electrolyte or gases, it let pass only the hydrogen ions. The work electrode is the cathode, a disk of 1 cm of radius and 0.7 cm of thickness is used for all the tests; the anode, as counter electrode, is a sheet of platinum area and a silver chloride electrode (Ag-AgCl) is employed as reference.

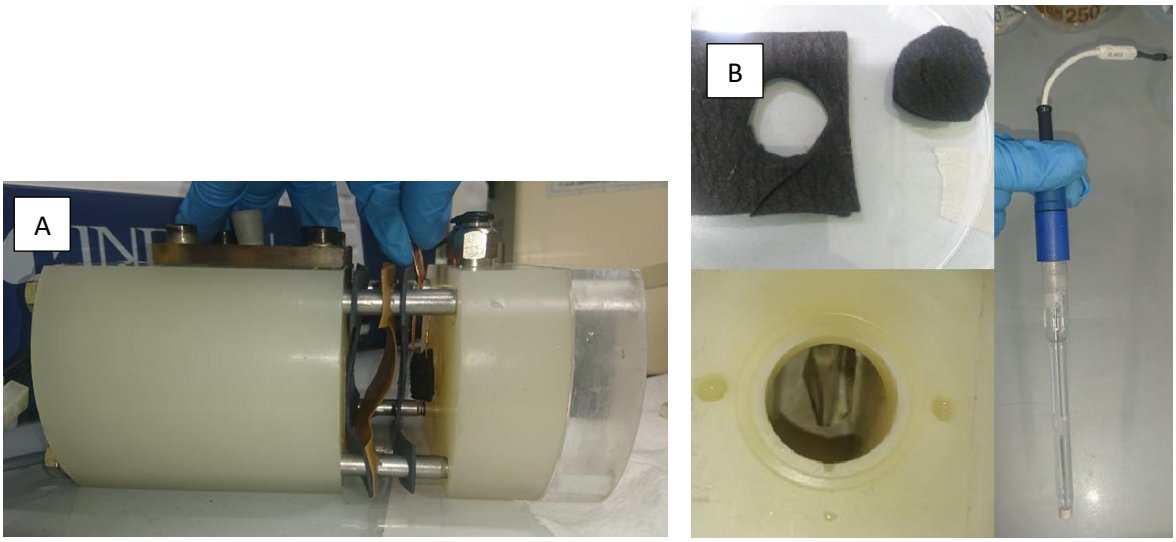

Figure 2.4. Experimental set-up used for the electrochemical test: A) Electrochemical cell and B) electrodes.

The reaction solution is recirculated for an external sealed flask with a volumetric pump (Gilson's MINIPULS Evolution, 40 ml/min), in the recipient the gasses are bubbled (N_2 and CO_2) and the line to the gas chromatograph is put, moreover there is also an arrangement to take the liquid samples without interfere with the process; the total volume of reaction between the cathode, the tubes and the recipient is of 60 ml; the bottle is placed in a hot plate (ARGO LAB M3-D) with controlled temperature through a sensor in an oil bath, such as to maintain the system temperature in 30°C.

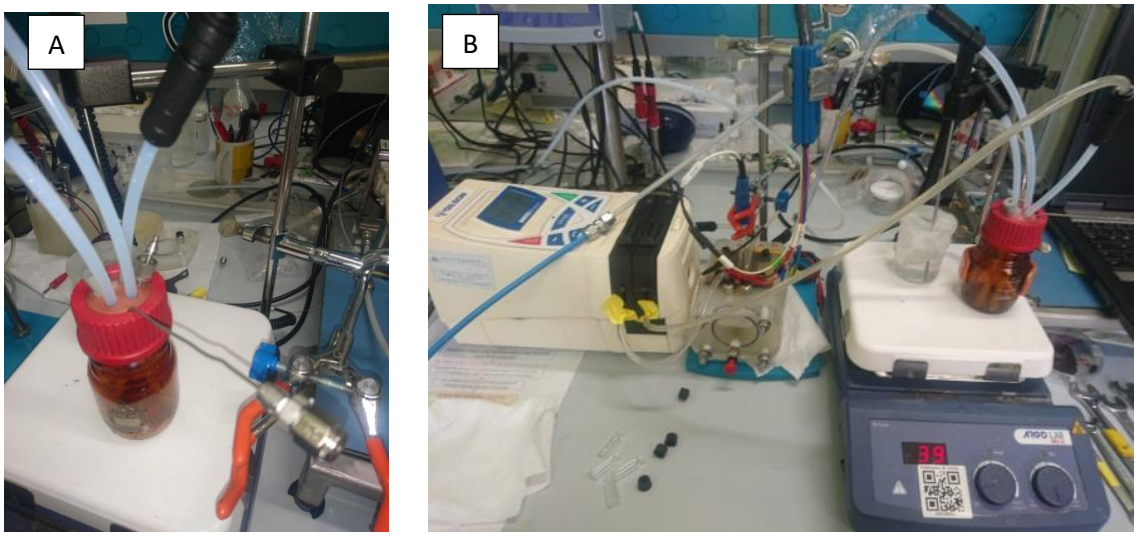

Figure 2.5. Experimental setup for the electrochemical test: A) External flask and B) Completely arrangement

- Instrumentation and software

The suitable potential is applied with a potentiostat (BioLogic SP-300), all the voltages are indicated between the cathode and the reference electrode. A line from the gas headspace of the external recipient is connected to the gas chromatograph (GC, Inficon Micro GC Fusion) to do measures in continue. Instead, liquid compositions are analyzed in discontinue, taking samples according to each test indication, in a high-performance liquid chromatograph (HPLC, Shimadzu) and gas a chromatography-mass spectrometry (GC-MS, Clarus 580). The carbon dioxide flow is controlled with a mass flow (EL-FLOW Bronkhorst).

2.2.2.2. Electrocatalytic protocol

All electrochemical tests are done with the procedure showed in the Figure 2.6. The system is saturated by flowing the corresponding gas for around 30 minutes before each cyclic voltammetry (CV) measurement. The CVs are repeated until obtaining a stable diagram of current in function of voltage. Samples of 2 ml of liquid are taken before and after this test.

While the carbon dioxide experiments are under way, the line to the GC should be already connected. It must be guaranteed the complete saturation to begin the CV and, even more relevant, the degasification first of the CA. The flow of CO_2 during the experiments is fixed at 8.86 Nml/min. During the Chronoamperometry (CA) experiments, 2 ml of solution are sampled each hour.

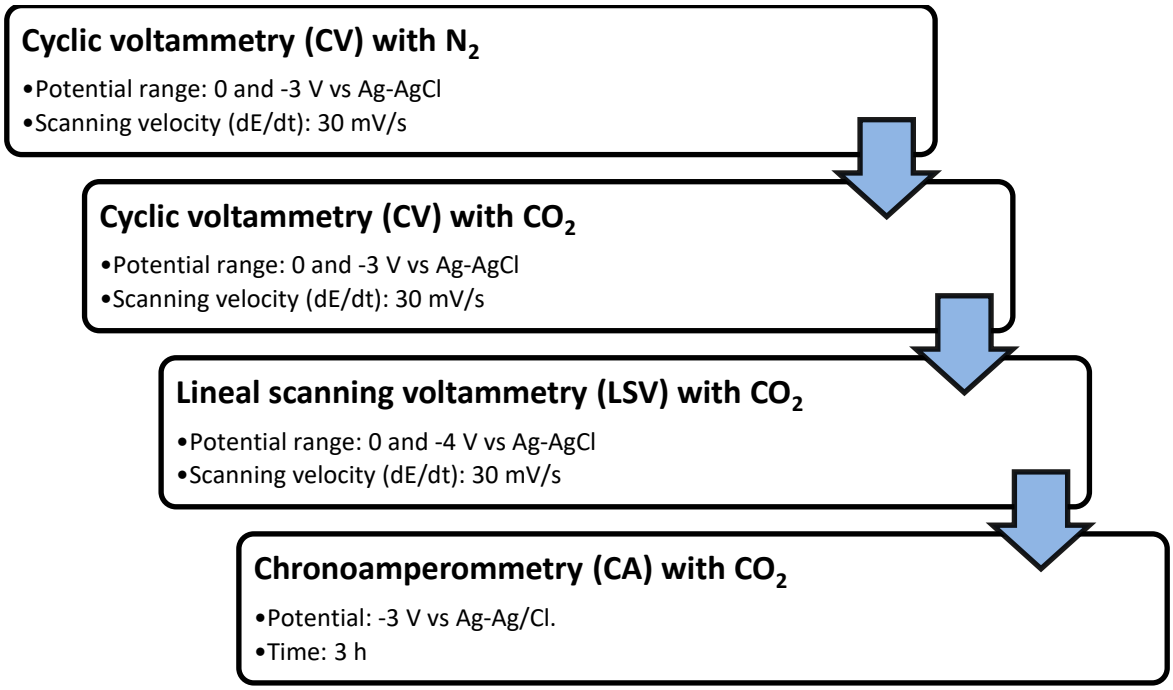

Figure 2.6. Electrochemical protocol used in the catalityc activity tests with their mainly conditions.

2.2.2.3. Electrochemical test

As indicated in the cell description, the cathode is a disc of carbon felt of 1 cm of radius and for each test different specifications of catalyst are used, these ones are summarized in the Table 2.5. For the electrodes with catalyst and enzyme the same methods of spray coated deposition (first step) and entrapment immobilization (second step), previously mentioned, are used. For all the experiments, in both chambers of the cell, potassium bicarbonate 0.1 M (KHCO3 – Aldrich, 99.7). In the presence of the enzyme, both in solution and immobilized, the cofactor NADH is added, replacing 1.5 ml of electrolyte by 1.5 ml of NADH (GERBU, >93%) solution 0.1 M.

Table 2.5. Electrochemical tests with specifications about the cathode. The kind of electrocatalyst and enzyme are indicated, also if the last one is immobilized in the same cathode or is added in solution.

Test	Support Material Pretreatment	Catalyst	Enzyme	Enzyme Form
1	No	SnO_2	None	None
2	Si	SnO_2	None	None
3	No	SnO_2	FDH	Solution (4mg/gCF/60ml)
4	No	SnO_2	FDH	Immobilized
5	No	None	FDH	Immobilized
6	No	None	None	None
7	No	Cu	None	None
8	No	None	ADH	Immobilized
9	No	Cu	ADH	Immobilized

3. Results and Discussion

3.1. Enzyme characterization results

3.1.1. Free enzyme activity

As was mentioned in the introduction section, in biological systems the dehydrogenase enzymes catalyzes with a higher priority the oxidation reactions and less frequently the reductions pathways of interest in this work. The specific activity of ADH and FDH enzymes, taken as references for the next experiments, have been calculated from the oxidation reactions of ethanol and formate, respectively. Furthermore, it has been said that NAD+ cofactor molecules are used and converted in NADH during the oxidation reaction, NADH, can be measured by spectrophotometric method at 340 nm to know how much reaction was carried out by the enzyme.

The Table 3.1 summarizes the slopes of NADH changes in function of time during one minute of reaction to calculate the specific activity of the ADH enzyme. In the search of a value with certain repeatability, the experiments were done with two different samples of a stock enzyme solution of 1 mg/ml, each one in turn diluted in two different ratios (1:100 and 1:200). For all dilutions, at least three or four analysis has been made and the slope measured was averaged and reported as the mean value.

Table 3.1. Specific activity results of free enzyme for 2 samples of a 1 mg/ml concentration of ADH enzyme, each one evaluated at two dilution levels with three or four repetitions at 30 ºC, in phosphate buffer 100 mM pH 7.5

	Enzyme Conc (mg/ml)	**Slope (Abs/min)**	**Mean slope (Abs/min)**	**Specific Activity (UI/mg_{enz})**
Sample 1	0.01	0.233	0.243±0.011	218.891
		0.254		
		0.251		
		0.235		
	0.005	0.122	0.122±0.000	219.567
		0.122		
		0.122		
Sample 2	0.01	0.265	0.275±0.009	247.163
		0.281		
		0.278		
	0.005	0.136	0.128±0.007	230.365
		0.123		
		0.125		

The enzyme activity highly depends on the different conditions of the reaction such as temperature, pH and reagents concentrations. Also, if during the experiments the efforts have been focused in using always fresh reagents and to maintain their temperature constant, the reaction toke place directly in a spectrophotometer cuvette where it was not

easy to work under controlled temperature conditions, so the enzyme properties was very variable. From each sample, it is expected a proportional relation between the ADH concentration and the mean slope found, which directly means an equal value of specific activity. In sample 1, the slope of dilution 1:100 is practically the double of the dilution 1:200 and, coherently, both specific activities differs only in 0.03%. Instead, for sample two, the difference has increased to 6.8%. Finally, to have a more representative value, an average of the four specific reported activities, 228.99 UI/mg_{enz}, will be used as a reference.

3.1.2. Immobilization

- Covalent bond immobilization

In the covalent-bond immobilization technique, the first step in the immobilization process is the functionalization of the carbon felt. The addition of aldehyde groups is desired; these come from the sequential hydrolysis and oxidation reactions of the epoxy groups added through the reaction of carbon felt with GPTMS. The quantity of aldehydes placed in the support is very important since the reaction between them and the lysine residues of the enzyme let the immobilization happen. The quantity of lysine residues should be enough to bind the major quantity of enzyme, but not so much to change its 3D structure.[26] Two different concentrations of the GPTMS, 0.5% v/v (22.64 mM) and 3.7% v/v (167.5 mM), have been compared to understand two different phenomena. First, if the concentration of the reactive influences the number of CHO groups per gram of support, and the second one, in the case of obtaining different aldehyde groups ratio, how it affects the immobilization parameters (specific activity and retained activity).

From the calculation described in Equation 2.2, 1.304 mmol and 1.895 mmol of aldehydes by gram of carbon felt have been found in the functionalized support with 0.5% and 3.7% v/v of GPTMS, respectively. Though the amount added to the second one is higher (of ca. 45 %), it is evident that the increment in the number of CHO molecules obtained per gram of support has not increased linearly with the amount of reagent, which does not even double. Bellow the immobilization processes are analyzed and the enzyme immobilized activities are measured. It can be seen that the immobilization efficiencies are higher for the experiments carried out with carbon felt with higher aldehyde content but, apparently, there is more retained activity by using 0.5% GPTMS in the pretreatment of the support, which indicates that the enzyme is subjected to denaturalization processes when the material has too much binding points.

Three different immobilizations experiments were done with each aldehyde-group concentration, 1.304 and 1.895 mmol/gCF, carbon felt. As presented in Table 3.2, different enzyme loadings were evaluated. The activity tests were performed with freshly prepared biocatalysts to avoid that the activity of the enzyme decreases due to storage conditions. In addition, with the purpose of not wasting materials, all tests were done in a small-scale experimental set-up. However, in this way, the weighing of the enzyme (of the commercial preparation) yielded to a high analytical error, and based on this, it was decided to increase the enzyme loading from 1 mg/g or 0.5 mg/g to 4 mg/g.

Table 3.2. ADH immobilization experiments. The quantity of carbon felt and the aldehyde groups contents are specified, as well as the reagents and the offered enzyme loading.

	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6
Carbon Felt (g)	0.44	0.44	0.185	0.58	0.55	0.49
Aldehyde groups (mmol/gCF)	1.304	1.304	1.304	1.895	1.895	1.895
HCO_3^- Buffer pH 10.05 (ml/gCF)	80	80	80	80	80	80
Enzyme loading (mg/g CF)	1	0.5	3	4	4	4
$NaBH_4$ (mg/ml)	0.5	0.5	0.5	0.5	0.5	0.5

For all experiments the immobilization process was followed by measuring the enzyme activity and the content of protein of the supernatant of the immobilization vessel surnatant. The samples were taken approximately every 20 minutes. The variation of the protein content was measured by means of the Bradford method. A decrease tendency of the protein quantity and the enzyme activity, as indication of the enzyme transference from the immobilization solution to the carbon felt, was expected. The results obtained in the first experiment (Exp 1) are not reported because the enzymes activities have showed from the initial time negative and zero values, indicating that no enzyme was present in a soluble form. This supposition was confirmed with the protein quantification since the initial concentration is far below of the prepared one and there is not a change of concentration during the process. However, considering these as possible errors in the immobilization monitoring, the activity of the immobilized enzyme was measured and will be discussed later.

For Exp 2, only the protein measurements were performed, obtaining immobilization efficiency (IE) around 32%; this value, together with the respective results of all other experiment, is reported in Table 3.3Table 3.3. Results of immobilization experiments with enzyme loading, immobilization efficiency and activity offered to the support.. The good results of experiments 4, 5 and 6 (respectively with IE of around of 77%, 69% and 57 %) have been mentioned during the functionalization. These tests correspond to the immobilizations performed with the material with the highest aldehyde content, so it has sense to observe higher yields. Another important factor is the enzyme loading. By comparing Exp2 and Exp3 where the offered enzyme loading increased from 0.5 to 3 mg by gram of support, respectively, an improvement of the immobilization parameters are observed.

Table 3.3. Results of immobilization experiments with enzyme loading, immobilization efficiency and activity offered to the support.

		Exp 2	Exp 3	Exp 4	Exp 5	Exp 6
Enzyme Loading	mg/gCF	0.120	0.651	3.285	0.763	0.813
IE	%	31.586	45.883	76.855	69.139	57.268
Activity offered	UI/ g CF	27.587	149.134	752.353	174.785	186.141

The high loading reached in the experiment 4 is remarkable, where 3.3 mg of protein by gram of carbon felt was obtained, in comparison to a maximum of 4 mg/gCF. In fact, it is important to point out that, also if the immobilization efficiencies of Exp 5 and 6 are not so bad, and the measured loading was the same, the initial protein measurements done by the Bradford assay revealed an initial concentration lower than expected, whereas in the experiment 4 the initial concentration found is very close to the theoretical added enzyme.

As an example of the immobilization process, the data of the experiment number 4 is shown in Figure 3.1 and Figure 3.2. The Figure 3.1 is the monitoring done by the specific activity test, this means that the surnatant samples are used as enzyme solution for ethanol oxidation and that the change of absorbance generated by the NADH production is measured. It is a qualitative way to know how much enzyme remained in the immobilization solution or, in other words, how much enzyme was not immobilized. The black curve is the sample taken at time zero, the pH of 10.05 is a very high pH for the ADH, so a blank sample, corresponding to a soluble enzyme subjected to the same conditions of the immobilization process but in absence of support, was reanalyzed every 20 minutes to have a reference of the decrease of activity caused by the pH and not by the decrease of the enzyme quantity. The red curve represents the samples taken at different immobilization times. A secondary axis ten times smaller was necessary to see the reduction of the values of the sample (supernatant). The measurements moved from a slope above 0.6 Abs/min to one lower than 0.01 Abs/min in just 20 minutes. From the experiments 3, 4, 5 and 6 a similar reduction has been seen, all with changes over 90%. The fast decrease of the activity of the supernatant indicates that the enzymes immobilize very fast on the functionalized carbon felt.

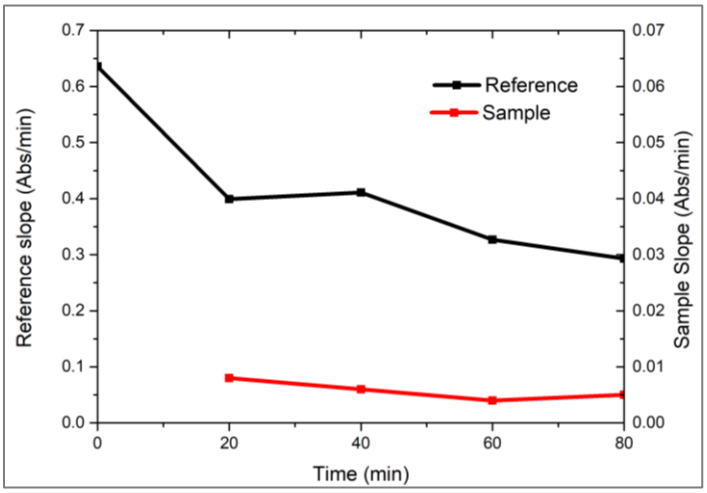

Figure 3.1. Monitoring of immobilization experiment 4 carried on carbon felt functionalized with 3.7% of GPTMS and with an enzyme loading expected of 4 mg/g CF: Absorbance slope in function of time of the immobilization solution supernatant (sample) and of a blank solution with only soluble enzyme as control (reference).

The Figure 3.2 correspond to protein mass changes and an analogous analysis can be done; there is a mark fall in the enzyme concentration from time zero to 20 minutes supernatant.

As described in methodology, the Bradford method values are used to calculate the immobilization efficiency, the results differ from the specific activity since the protein results consider the physical mass quantity of the enzyme, while the enzyme activity is a way to measure the quantity of enzyme that is able to carry the reaction. From both tests, it was concluded that the immobilization time can be set at 40 minutes. Above this time, no more enzyme is attached to the support and a fast decrease of the activity of the reference is observed. Thus, in order to preserve the enzyme activity on the support, the immobilization time should not continue over 40 min.

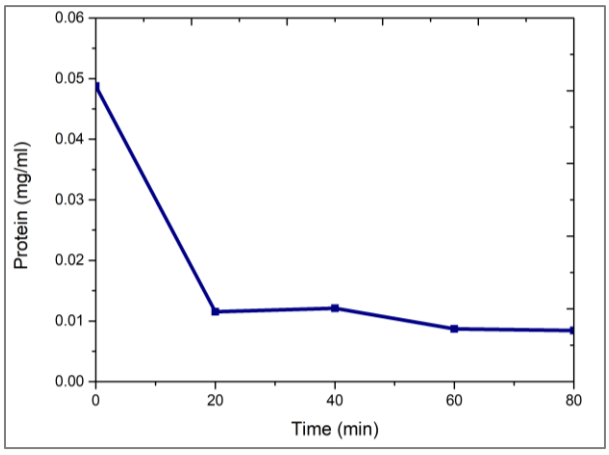

Figure 3.2. Monitoring of immobilization experiment 4 carried on carbon felt functionalized with 3.7% of GPTMS and with an enzyme loading expected of 4 mg/g CF: Protein concentration of the immobilization solution supernatant measured by Bradford method.

- Entrapment immobilization

The second method of immobilization studied was simpler that the covalent one because requires less time and no functionalization should be done. However, one disadvantage is that after the preparation and application of the immobilization solution there is no way to monitor the process, it should be supposed that all the solution remains in the carbon felt. In this sense, the quantity of protein has been calculated to be 3 mg of enzyme by gram of carbon felt, this value will be considered as the real enzyme loading and, consequently, the activity offered to the material have been calculated to be 694.47 UI/gCF. The performance of the enzyme will be evaluated from the activity measurements, but securely, taking this overestimated reference value that indicate an immobilization efficiency of 100%, will generate lower retained activity values than the real ones, as can be seen from the equation Equation 2.5 where retained activity is calculated from the activity measurement divided the activity offered.

3.1.3. Immobilized enzyme activity

- Covalent bond immobilization

Initially, 10 mg of immobilized carbon felt were weighted and put in ethanol in presence of NAD+ at the suitable conditions of reactions (pH 7.5 and temperature of 30ªC). At determined reaction time the test is stopped and a sample of surnatant is taken to measure

the NADH absorbance at 340 nm. To calculate just one enzyme activity value, it was necessary to measure the increase of NADH absorbance in a discontinuous mode, so NADH absorbance is measured in different experiments at three or four different reaction times. To guarantee a certain repeatability, for each reaction time the procedure was done in duplicates or triplicates. The control of suitable conditions for the functioning of the biocatalyst is very important, but additionally, a high precision in the sampling time is required. It is not an easy work by taking into account that the carbon felt fibers need to be separated to avoid their interference with the spectrophotometer measurements. In this way it is expected to find a linear behavior between the NADH absorbance and the reaction time, the slope of this linear relation is used in the Equation 2.3 to calculate the immobilized enzyme activity.

This method was used to calculate the enzyme activity after the immobilizations Exp 1, Exp 2, Exp 3 and Exp 4. With the carbon felt immobilized in in the first experiment (Exp 1) 18 tests of activity were carried out, each one evaluated at 1, 3 or 5 minutes; values between 0.011 and 0.556 absorbance units have been found and just two results have been discarded since showed negative numbers. However, the results have not presented any kind of dependence with time, on the contrary, they show a random behavior. The same tendency was found in the carbon felt of "Exp 4", but this time the reactions were stopped at 0.5, 1 and 2 minutes to see if the linear expected relationship occurred at shorter times, but the results were also incoherent. Only for the immobilized carbon felt of "Exp 2", the measurements showed a relation between reaction time and the NADH concentration, which is plotted in Figure 3.3. Absorbance measured after of 1, 3 and 5 minutes of ethanol oxidation reaction with carbon felt of immobilization experiment 2 (0.12 mg of enzyme by g of CF). . Although it was not possible to calculate the slope required to quantify the enzyme activity, it is very important to remark that these, and all positive values find in the activity measurement, are a clear indication of the presence of the reduced form of the NAD+, so an evidence of immobilized enzyme carrying out the ethanol oxidation reaction. The absorbance measurements obtained from the carbon felt of immobilization 3 have been discarded because most of them were negative and zero values.

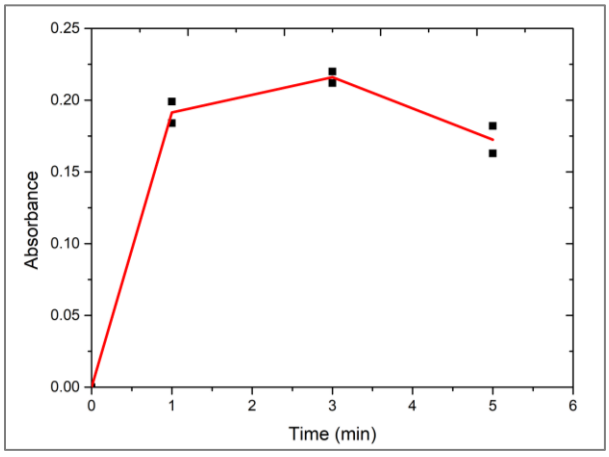

Figure 3.3. Absorbance measured after of 1, 3 and 5 minutes of ethanol oxidation reaction with carbon felt of immobilization experiment 2 (0.12 mg of enzyme by g of CF).

A hypothesis to explain the abovementioned results for the enzyme immobilization activity

is the possible heterogeneity in the enzyme distribution on the support. Each repetition, in each reaction time, is done with a new sample of 10 mg of immobilized carbon felt. Even if it has been taken mixing fibers of different points of the material, it is still very possible not to have a similar enzyme quantity in each test. In subsequent tests, a higher amount of carbon felt with supported enzyme was used (i.e. 0.1 g) and the samples were taken every minute to verify the increment in the NADH concentration. For the carbon felts of immobilizations Exp 5 and Exp 6 no coherent results were observed, in fact, almost all measurements had negative or zero absorbance values. It should be remembered that these immobilizations were done in the carbon material with the higher content of aldehydes groups, so the not possibility to measure any signal of enzyme activity can also be a confirmation of the protein denaturalization generated by an excess in CHO binding points.

- Entrapment immobilization

Also in this case, both experimental set-ups to calculate the immobilized enzyme activity have been tried (10 mg CF based method and 0.1 g CF based method). It should be said that some experiments have resulted in the same imprecise tendency of covalent bond immobilization. For example, a very similar behavior to the one described in Figure 3.3 have been observed in a test done for the approach based in 0.1 g of immobilized carbon felt with a proportional increased of the reaction volume; absorbance measurements were taken until 6 minutes with all results around 0.2 absorbance units. On the other hand, the next two proves are highlighted to present the linear tendency searched and, practically, the same enzyme activity, even if each one has been evaluated by a different kind of experimental set. As can be seen in Figure 3.4 both have a slope around of 0.009 absorbance units per minute, consequently, both present an enzyme activity of 0.4 UI/gCF and, in reference of the activity offered to the support (694.47 UI/gCF), just 0.058% of retained activity was observed. These values cannot be taken as an indication of bad performance of immobilized enzyme, because as have been mentioned, the enzyme also has expressed, in short times, high absorbance values. On contrary, it is clear that the entrapment method showed more homogeneous effects than chemical immobilization approach.

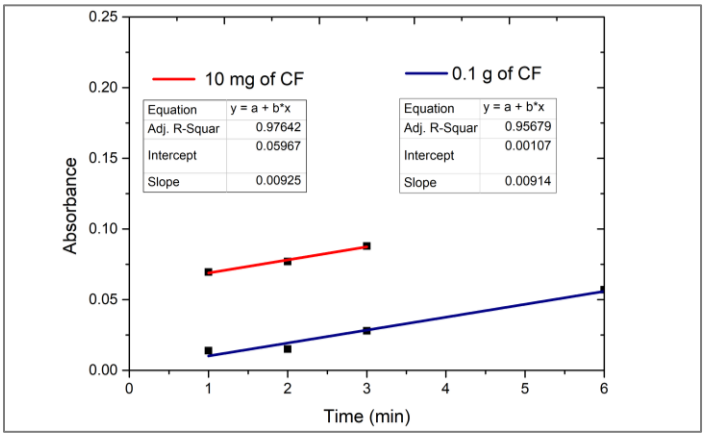

Figure 3.4. Absorbance measured at different times in ethanol oxidation reactions with carbon felt immobilized by entrapment technic (3 mg/g CF). Red: 10 mg of carbon felt based test, each point corresponds to a mean value of two experiments stopped at the respective reaction time. Blue: 0.1 g of carbon felt based test, all samples taken from the same experimental set.

Another important evaluation factor to compare the immobilization technics is the compatibility with the deposition of the inorganic electrocatalyst, which employs a spray coating procedure of an ink of the metal oxide dispersed in a solution of Nafion (used as binder) and isopropanol (used as solvent). Indeed, it is necessary to find a logical order to place both, electro- and bio-catalysts, in the support. In the case of the chemical immobilization, if the inorganic catalyst is first deposited in the carbon felt, it can be removed by agitation during the fixation of the enzyme; on the other hand, if the enzyme immobilization is done first, it will be subjected to heating over 100°C that is required after the metal oxide deposition, with a consequent denaturation of the enzyme. For these reasons, the entrapment immobilization method, that it compatible with the electrocatalyst deposition, is the better option to the next electrocatalytic experiments with the hybrid systems.

3.2. Electrocatalysts Results

The electrocatalytic activity results are described below. Nine experiments were performed following the electrocatalytic protocol indicated in Figure 2.6 in each case a different catalytic system (cathode) was used. Their catalytic performance in the CO_2 reduction was evaluated by using parameters as generated current density, H_2 production, liquid products quantification and electrochemical stability.

The nine tests are composed by a) one blank test, where a carbon felt without treatment or catalyst was tested; b) two tin oxide electrodes, one in a thermally treated CF support and one in the CF raw material, looking for identify if the hydrophobic nature of the carbon felt has some influence in the process, as well as, if the CF pretreatment can improve the catalyst deposition and, in turns, the catalyst performance; c) a copper catalyst, pretreated to obtain a higher ratio of metallic and oxides quantity; d) two enzyme-immobilized electrodes, one with the formate dehydrogenase (FDH) and the other one with alcohol dehydrogenase (ADH), it is important to point out that both enzymes carried out the reactions by an electrochemical transference of electrons with NADH cofactor, also supplied in the system, so these experiments are expected to be useful to observe the behavior of the enzyme during the conditions required by the electrocatalyst, conditions at which will be subjected in the hybrid catalyst system; e) the last two electrodes are the hybrid bio-electrocatalytitc system, the FDH is coupled with the tin oxide and the ADH is coupled with the coper catalyst.

3.2.1. Electrodes preparation

In section 2, the procedure to prepare the catalyst ink for electrocatalyst deposition is described in the section 2, all calculations were made considering a big percentage of losses (70%), which include, among others, the catalyst dispersed in air, the remaining catalyst in the ink recipient, in the spray gun and in the mask used to delimit the shape and size of the electrodes. However, the efficiency of deposition was higher-than-expected, as it is presented in Table 3.4. The four tin oxides electrodes show a very repetitive efficiency, all around 53% and 50%. Instead, both copper preparations differs a little, the electrode with only inorganic catalyst, has an efficiency of around 44%, whereas the one with the immobilized ADH is around 32%, it is coherent with the experimental experience since it

was more difficult to get a homogenous dispersion with the Cu than with the SnO_2, the second one was easier and more homogeneous.

The hybrid electrodes were prepared in the following mode: firstly the electrochemical catalyst was deposited and the biocatalyst secondly, so the enzyme immobilization does not influence the catalyst deposition. On contrary, one future point of study would be the effect of the presence of metals and oxides materials in the carbon felt at the moment of the fixation of the protein. As it has been explained, the Nafion entrapment have not been monitored or evaluated, so in this work, it is considered that all contacted enzyme was placed on the electrodes, thus it is expected to have 4 mg of enzyme by gram of carbon felt.

Finally, it should be said that by the quantity of deposited SnO_2, it is not observed an important difference between the raw carbon felt and the pretreated one. Actually, it can be inferred from the electrochemical performance, that there is a different distribution of the catalyst on the carbon felt support, which yielded to higher diffusional limitations in the case of the treated one. Therefore, the carbon felt without the calcination pretreatment was chosen for the tests of the hybrid systems and to deposit the Cu catalyst.

Table 3.4. Loadings of inorganic catalysts and enzyme in the carbon felt support achieved with spray coating deposition and Nafion entrapment immobilization, respectively.

	Catalyst loading	Enzyme loading
	mg/gCF	mg/gCF
Carbon Felt not treated (CF_{nt})	-	-
Carbon Felt treated (CF_t) + SnO_2	5.294	-
CF_{nt} + SnO_2	5.065	-
CF_{nt} + Cu	4.400	-
CF_{nt} + FDH_{Imm}	-	4
CF_{nt} + SnO_2 + FDH_{Sln}	5.134	4
CF_{nt} + SnO_2 + FDH_{Imm}	5.042	4
CF_{nt} + ADH_{Imm}	-	4
CF_{nt} + Cu + ADH_{Imm}	3.254	4

3.2.2. Electrocatalytic Activity

- **Electrocatalysts Cyclic Voltammetries**

Each test started with a cyclic voltammetry (CV) in nitrogen conditions. For each electrode, the number of cycles done was different, which depended on the cycles necessary to reach a stable condition. At this point, it can be assumed that the redox reactions are not more associated to the transformation of the electrode itself but to the reactions at the electrode surface (related to the desired carbon dioxide reduction or to the HER). The Figure 3.5 presents as an example, the complete CVs done by flowing nitrogen in the medium for the carbon felt electrodes only with SnO_2 or with the FDH enzyme. In the SnO_2 electrode

diagram (Figure 3.5A) some cycles have been remarked to observe the changes suffered by the electrocatalyst system; between others, in cycles 5 and 8 a coupled redox reaction can be seen, these ones increase and decrease again between initial and final curves, cycle 1 and 15 respectively. The low activity expressed by the enzyme in nitrogen environment (Figure 3.5B) will be discussed later, but the whole CVs, and specifically the zoom done in both electrodes curves, are showed to remark the noise characteristic of the instability and complexity of biological systems.

Henceforth, only the last sequence found to each test will be illustrated to identify and to analyze the meaning and relevance of the current peaks, the faradic current magnitudes and the different behavior of electrodes, between the absence of carbon dioxide and the saturation with this greenhouse gas. Also, unless otherwise noted, all potentials will be indicated in reference to Ag/AgCl electrode.

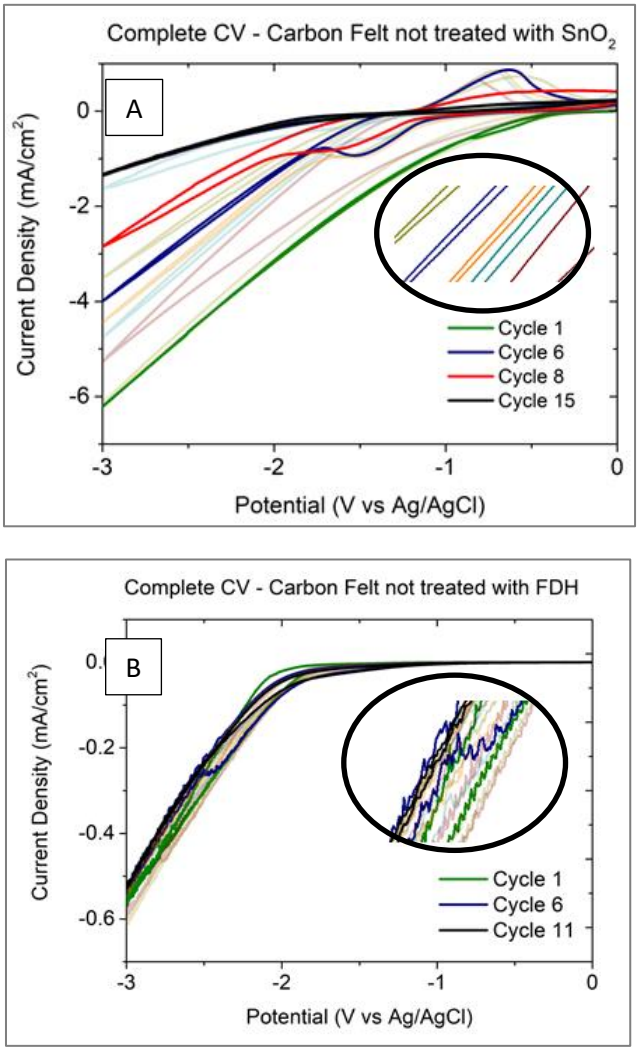

Figure 3.5. Complete CV tests with A) SnO_2 catalyst deposited and B) FDH enzyme immobilized, both, in raw carbon felt based electrodes, in N_2 saturated 0.1 M $KHCO_3$ aqueous solution at a scan rate of 30 mV s^{-1}. Insets correspond to the zoom of CV curves between -2.25 and -2.50 V

The Figure 3.6 shows the CVs made with the not treated carbon felt electrode and without catalyst. From 0 to -1.5 V the curves obtained with both gases are very closed; in this range where neither hydrogen evolution reaction (HER) nor CO_2 reductions occurs, the carbon felt let see an oxidation and reduction coupled peaks that probably are related with the own support changes, since as can be find in literature, carbon fibers can be oxidized with the addition of hydroxyl and carboxylic groups in surface with electrochemical methods.[32] Furthermore, respect to standard hydrogen reference, the range of cathode potential scan begins in positive values, giving certain oxidative environment at the initial part of the sweep (0.2 V vs SHE). At more negative potentials the reduction current increases and both curves slightly come apart; with carbon dioxide pre-saturation this can indicate certain catalytic activity of the own support to the CO_2 reduction, however, the higher values obtained with the nitrogen atmosphere test indicates a mainly correspondence with HER reaction,

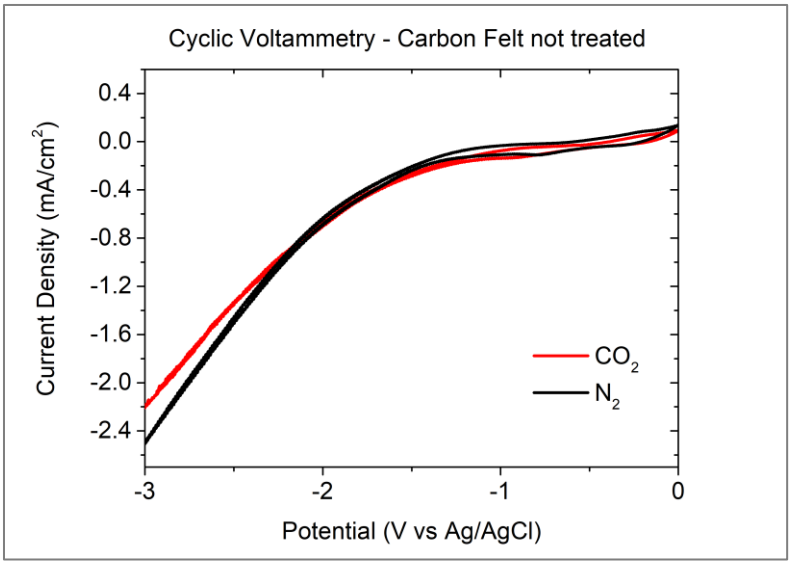

Figure 3.6. Last cycle of CVs on raw carbon felt in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}.

As mentioned before, one of majors problems found in literature of the support material is its hydrophobicity, therefore, the electrocatalytic activity of the tin oxide deposited in the support, with and without a thermal modification, was evaluated to decide the carbon support used in the following electrodes. The thermal treatment done was chosen because it is the simpler one and it is reported to increase the electrical conductivity, the active sites number and the hydrophilicity, resulting in a better electrochemical behavior of carbon felts.[26]

The Figure 3.7.A and the Figure 3.7.B are the CV's obtained with the SnO_2 supported in the CF material when it is and is not treated, respectively. The CF_t+SnO_2 electrode has a very linear behavior when the electrolyte is saturated with N_2. Furthermore, there is an important and particularly separation between the increase and decrease curve of the potential scanning, which indicates a higher capacitive behavior of this electrode. Instead, in CO_2 saturated environment, the potential applied generates very low currents compared with all other experiments. The CF_{nt}+SnO_2 electrode has a similar behavior to the carbon

felt blank; both curves, in N_2 and in CO_2, show one oxidation and one reduction peak between 0 and -2 V, at higher potentials the reduction currents increase, and finally, higher values are obtained in the inert gas than in carbon dioxide; however, in both CVs the current densities are approximately the half of that showed in the test of the CF_{nt} (raw support material without catalyst).

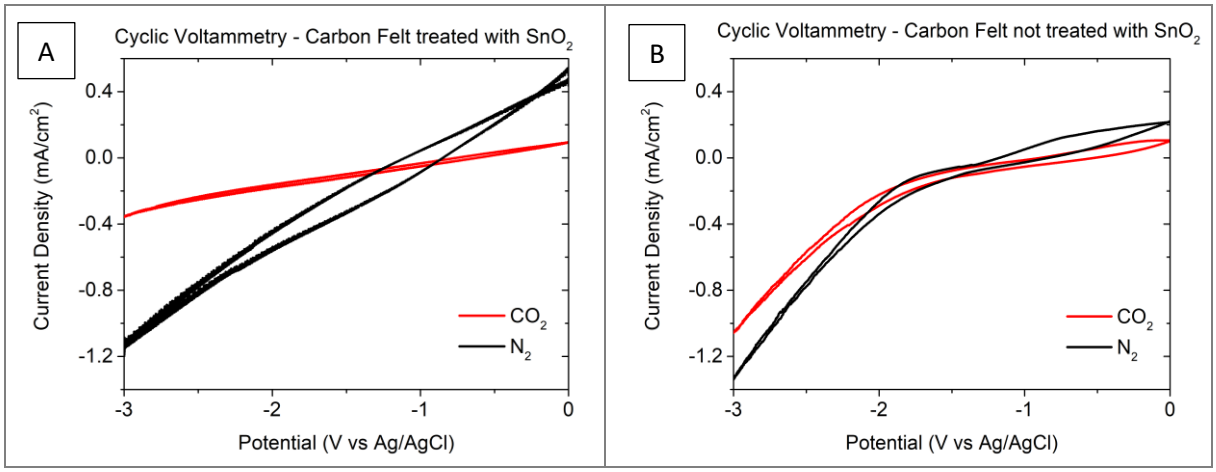

Figure 3.7. Last cycle of CVs on two electrodes in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}: A) CFt+SnO_2, B) CFnt + SnO_2.

The lower currents observed with the CF_t+SnO_2 electrode than with the CF_{nt}++SnO_2 in CO_2 saturated electrolyte, are supposed to be related to a lower catalytic activity. As the deposition seems to have been made correctly with a calculated SnO_2 loading of 5.29 mg/cm^2, it is concluded that the modification generated by the calcination is interfering in some other way with the CO_2 reaction on this electrode. One hypothesis is that making more hydrophilic the carbon felt, the catalyst ink have penetrated deeper during its deposition in the support. Since the carbon felt has a considerable thickness (i.e. 7 mm), so the SnO_2 is provably highly distributed in the bulk and not only superficially. In this way, with the N_2 flow the HER has a lower limitation because the aqueous electrolyte can also penetrate easily in the hydrophilic electrode, instead, apparently when the CO_2 is added there is some kind of mass transfer limitations. This observation will be evaluated again when the current and hydrogen profiles over the time are shown. Other possible consequence of the thermal treatment is the non-faradic behavior showed in the current-potential curve under N_2 flow. This support is widely studied as electrode, but also as capacitor material, and some researches have reported the increment in the capacity of carbon felt to store energy after thermal modifications,[33] which explains the here observed behavior.

In the experiment with the CF_{nt}+SnO_2 electrode the initial redox coupled reactions can be representative of both, support and tin associated reductions and oxidations. it is supported since the blank had similar signals but also because the different tin based catalyst researches describe two oxidation peaks related to metallic Sn oxidation to Sn^{2+}, and Sn^{2+} oxidation to Sn^{4+}; and just one reduction reaction of these two species, due the scan rate of the CVs (in the literature references have been of 10 and 5 mV/s^{-1}) is possible to obtain

both oxidations together. Here, the HER reaction seems to prevails as well by observing the higher currents in the curve under N_2 flow, but as in both curves the currents have decreased in reference to the CF_{nt} test, it is necessary to verify with the other electrochemical test if only hydrogen reduction have been inhibited. This would mean that even with lower current densities, the desired reaction could be occurring, or instead, in general the catalyst is not working properly, and all reactions are being decreased in an equal manner. [13][34]

The second catalyst studied is copper, CF_{nt}+Cu, its CVs are presented in Figure 3.8. The early part of the scan shows the oxidation and reduction peaks, mainly in the nitrogen saturated bicarbonate solution. On difference to the previous catalyst, the reduction reactions look more significant than the oxidation one. It agrees with theory because the standard oxidation potential of copper is 0.34 V vs SHE , while for tin is 0.14 V vs SHE. As have been said in the introduction section, multiple studies have been made regarding the ratio of metallic and oxide catalysts to improve the expressed activity; in this way, looking to the generation of some percentage of oxide groups, the cupper used was subjected to a calcination, this means that there are reducible groups that may be reacting. Moving on, from -1.5 V vs. Ag/AgCl a reduction reaction is observed, it should be noted, that in nitrogen, the currents reached are more or less the same of raw carbon felt, whereas in carbon dioxide environment they are lower than those of the bare support material, but grater that the ones with the SnO_2 electrode. Consequently, a higher catalytic activity is anticipated.

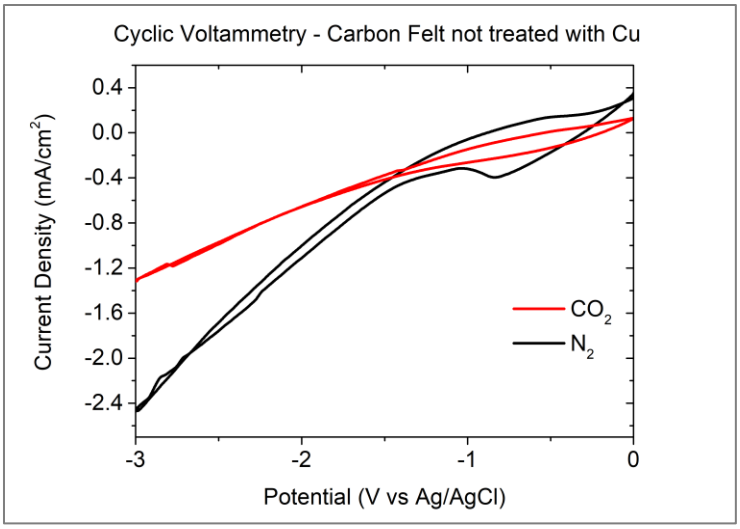

Figure 3.8. Last cycle of CVs on CF_{nt}+Cu electrode in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}.

- **Electrocatalysts Linear Sweep Voltammetry**

After the cyclic voltammetries, a linear sweep voltammetry (LSV) was done with a continuous flow of carbon dioxide; the potential was varied from 0 to -4 V vs. Ag/AgCl with the same scan rate of the CVs, 30 mV/s. This test is used as a representation of the initial state of the catalyst system before the chronoamperommetry (CA). It is useful to identify the density currents that can be obtained, and the onset of the reactions observed.

In the Figure 3.17 the LSV results for the raw carbon felt electrode and the three electrodes with electrochemical catalyst (CF_{nt}, CF_t+SnO_2, CF_{nt}+SnO_2 and CF_{nt}+Cu) are presented. Of all the procedures, the first experiment made was on CF_{nt}+SnO_2, from its LSV information and the measurement of the electrolyte resistance reported by the potentiostat, the real electrode potentials at -1, -2 and -3 V was calculated, Equation 3.1 and Table 3.5 . The CA voltage chosen was -3V, because it would permit to have the major possible currents but also to have not E_C over -1.8 V vs. Ag/AgCl, that is the maximum value used in literature with SnO_2 electrodes.[13]

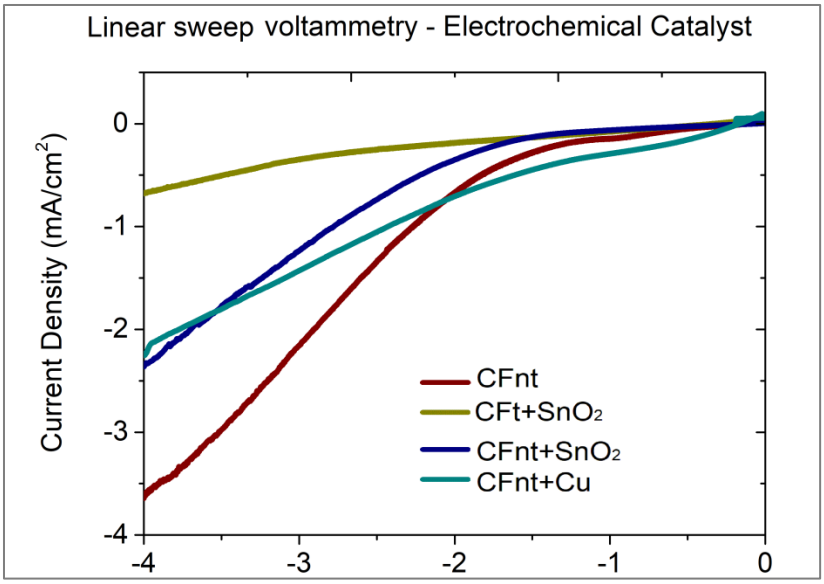

Figure 3.9. Linear sweep voltammetry on 4 electrodes in CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s-1.

$$E_C = E_{ref} - R_{electrolyte} * I \qquad \text{Equation 3.1}$$

Table 3.5. Electrical measurements on LSV analysis with CFnt+SnO_2 for real cathode potential calculations.

E_{ref} (V)	$R_{electrolyte}$ (Ohm)	I (Amp)	Ec (V)
-1	362.0	-0.0002	-0.928
-2	350.1	-0.0011	-1.615
-3	317.3	-0.0039	-1.771

The linear sweep voltammograms confirmed the information observed in the CVs; the blank electrode presented the major currents, with a onset reduction potential of -0.8V vs. Ag/AgCl, the CF_{nt} + SnO_2 electrode had a greater activity than the CF_t + SnO_2 one (using the treated support) and, at almost all potentials, the copper evidences a higher electrocatalytic activity that the tin oxide-based electrodes. For all cases the more pronounced onset is around -1.5 V vs. Ag/AgCl, coincident with the literature reported potential for the hydrogen evolution reaction. In fact, for tin oxide catalysts is difficult see the other changes of slope in the diagram; on CF_t + SnO_2 there is a very small change in the curve, around -3.25 V vs. Ag/AgCl, and in CF_{nt} + SnO_2 there is another one around -2.3 V vs. Ag/AgCl; both potentials after of be corrected with the electrolyte resistance are

voltages values closed to the -1.6 and -1.7 V vs. Ag/AgCl, respectively, range that has been reported in other investigations to the formate production (from -1.4 to -1.8 V vs Ag/AgCl). At least, it is remarkable that after the nitrogen and carbon dioxide CVs, the reduction of the catalyst is almost inexistent, where the first signals were observed, between 0 to -1 V vs. Ag/AgCl, now the plots are flat.[20]

Copper catalysts have been widely studied, with different forms, geometries and dimensions. There are researches with sixteen different carbon compounds generated by copper during the CO_2 reduction reaction. It could make more difficult to specify the onset potentials. Some examples are the CO production in Cu nanowires and the synthesis of ethylene and methane in Cu nanocubes catalysts, both in CO_2 saturated 0.1 M $KHCO_3$ aqueous solution. The most repeated value found in literature as CO_2 reduction onset is -1.1 V vs. Ag/AgCl, which could be agree with a slight change obtained in the cupper curve of Figure 3.9 first of the hydrogen reduction. On the other hand, there are also papers that present CO_2 reductions onsets at around -1.5 V vs. Ag/AgCl, which are overlap with RHE onset. [21] [22] [15]

- **Electrocatalysts Chronoamperommetries**

Finally, a chronoamperommetry is done, all catalyst were subjected to -3 V vs. Ag/AgCl for three hours, to quantify the current density generated as an indicator of the reduction reactions obtained. In all the experiments, a micro gas chromatograph (micro-GC) was used in continuous to analyze the gas products. Only hydrogen was detected, the flows measured are presented in Figure 3.11. For the liquid products, a high-performance liquid chromatograph (HPLC) and a mass spectrometer coupled to a gas chromatograph (MS-GC) were used. It is worth noting that the micro-GC analysis were stopped at the same time of CAs. Taking into account that a certain quantity of H_2 should remain in the headspace of the external recipient of the experimental set, and considering that the degas would depend only of the assembly geometry, one experiment has been was made to measure the trend of H_2 outgassing from the system, until zero hydrogen concentrations. Based on this result, the last part of the H_2 flows of the other curves have been modeled. In Figure 3.10, the fit of the base measurement is shown.

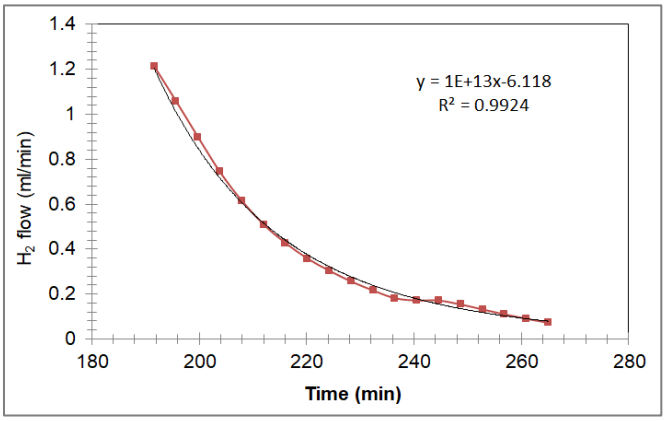

Figure 3.10. Potential fit of hydrogen degas flow after of a chronoamperommetry with carbon felt not treated electrode with immobilized ADH.

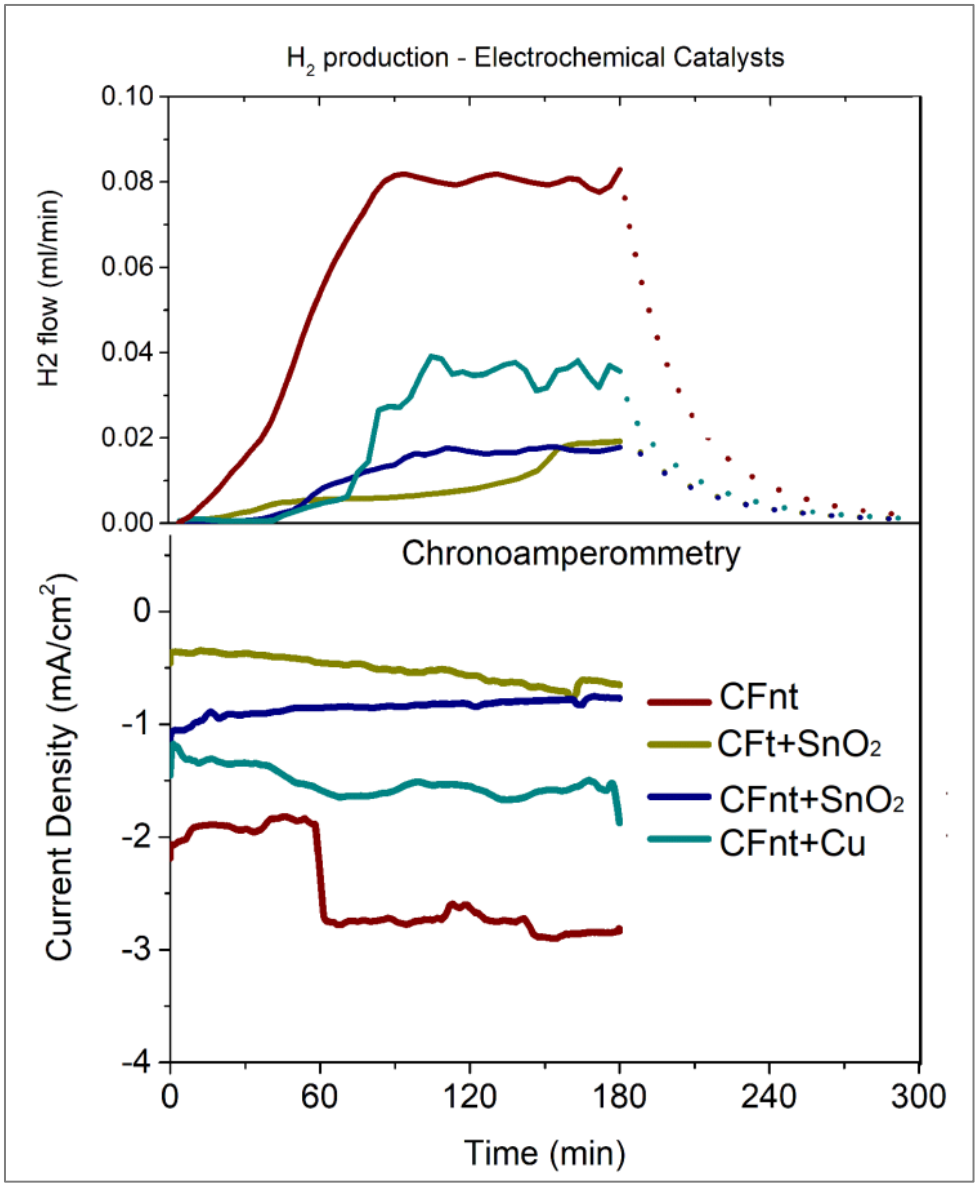

Figure 3.11. Hydrogen volumetric flow (upper graph) measured with a continuous gas chromatograph during the chronoamperommetries (lower graph) on 4 electrodes in CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at -3 V vs Ag/AgCl. The last part of H_2 flow diagrams (dot line) have been modeled instead of measured.

The experiment with CF_{nt}+SnO_2 electrode showed a good stability since the current density of the CA test was almost constant around -1 mA/cm^2, coherently with the LSV diagram where -1.2 mA/cm^2 were produced at an applied potential of -3 V vs. Ag/AgCl. This is also reflected in the micro-GC analysis, the hydrogen production, after of a dead time of 40 minutes, reaches a steady value around 0.02 ml/min until the end of the reaction at 180 minutes, at this point, two hours of degas of the reactor has been modeled (dot line). Contrary to the theorical references, the CA and micro-GC tests with the CF_t+SnO_2 electrode did not show a better electrochemical behavior, and it needed more time to arrive at the same stable conditions of the CF_{nt}+SnO_2. Furthermore, it would be thought than the

lower production of hydrogen is good, but it is possible that it just corresponds to lower current densities; thus, the faradic efficiencies have to be compared to draw conclusions.

One possible explanation of the delayed activity of the CF_t+SnO_2 electrode would be mass transfer problems; the calcination of the support was expected to reduce the hydrophobicity and improve the deposition of the catalyst, but the catalyst loadings in the CF treated and not treated were practically the same, (5.29 and 5.06 mg/cm^2 for the CF_t+SnO_2 and CF_t+SnO_2, respectively). An explanation could be the different catalyst distribution within the carbon felt; indeed, the catalyst ink most provably penetrates more (in the thickness of 0.7 cm) in the treated material due to its hydrophilicity, and thus the carbon dioxide needs more time to arrive to the tin oxide surface.

From the LSV the copper is projected to produce more or less -1.4 mA/cm^2, slightly higher activity than the CF_{nt}+SnO_2, it value have been observed at the beginning, but the reactions continue to increase a little till 60 minutes, where the current density stabilizes at around -1.7 mA/cm$^{2.}$ Nevertheless, at this time also grows the H_2 concentration in the micro-GC , achieving a H_2 flow over 0.03 ml/min.

It should be said that the blank electrode experiment (CF_{nt}) in Figure 3.11 have showed some anomalies, later confirmed by an H_2 Faradaic efficiency over 100%. The presented curve is an indicative normal behavior; it is supposed from the CA curve obtained and based in the stabilization tendency of the hydrogen production seen in all others electrodes. The chronoamperommetry let see that the CF_{nt} experiment corresponded to the expected current density of the LSV at -3 V vs. Ag/AgCl for 60 minutes, then, a sudden rise of current density has appeared carrying it at about -2.5 mA/cm^2 (the LSV value), at this point there is a coherent micro-GC response, which means that also the H_2 outlet of the system has gone up, coming close to 0.1 ml/min.

- **Enzyme and Hybrid system Cycle Voltammetries**

The cyclic voltammetries with CF_{nt}+FDH_{Imm} electrode, Figure 3.12, show a very good electrochemical activity. The CV curves show very low current densities with the N_2 saturated test in contrast to the very fast and high reduction currents observed with the CO_2 environment. It could mean a very big preference for the carbon dioxide reduction. Besides, seems that there are two different pronounced reactions, one at about -1 V vs. Ag/AgCl and another at around -1.5 V vs. Ag/AgCl, that could be related to the HER and CO_2 gas reduction reactions. A very different behavior is showed by the CF_{nt}+ADH_{Imm} cathode, Figure 3.13. There is one important observation about these curves, which present characteristics very similar to the blank electrode experiment, but with higher currents; both curves, in N_2 and in CO_2, have very similar magnitudes and behavior, at low potentials there are redox reactions that can be attributed at the own support reactions, and it seems to be an increment effect in the properties of the material in terms of capacitance. In this way, also if after -2 V vs. Ag/AgCl the system seems to prefer the CO_2 reduction, it is very possible that the high currents will be just correspondent to the HER because the ADH enzyme is not specific for the CO_2 reduction reaction.

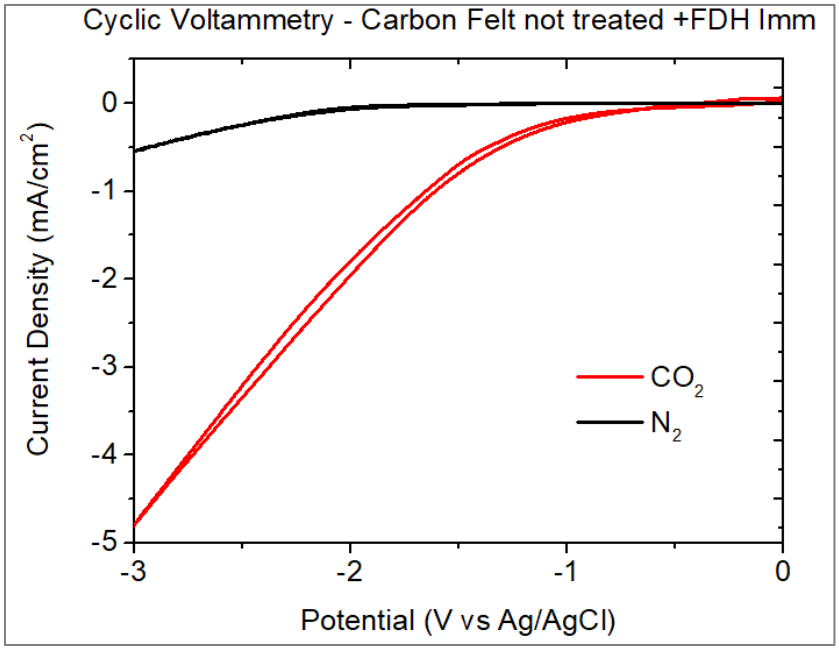

Figure 3.12. Last cycle of CVs on the CF_{nt} + FDH_{Imm} electrode in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}

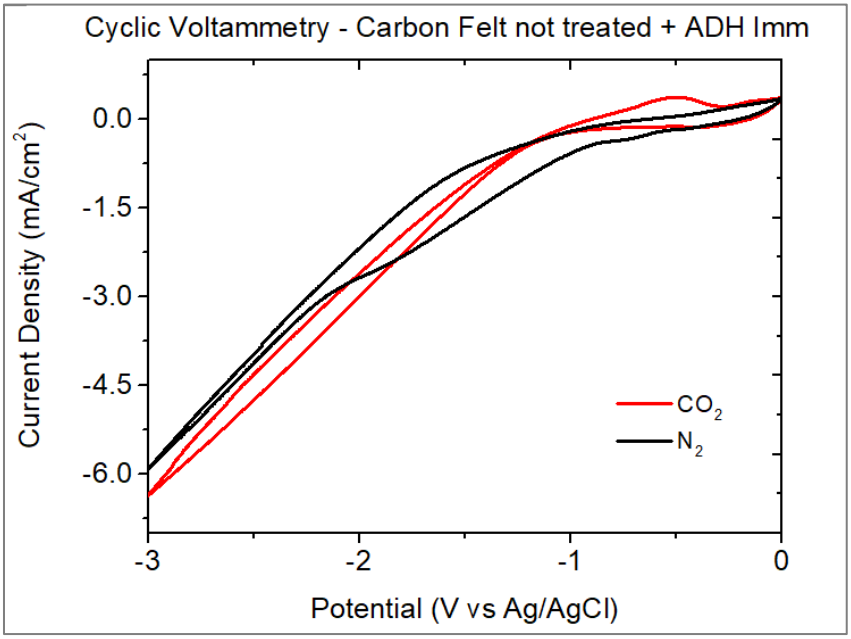

Figure 3.13. Last cycle of CVs on CF_{nt}+ADH_{Imm} electrode in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}

In literature, two kind of bio-electrode mechanisms have been described. One possibility is the direct transport of electrons of the cathode to the enzyme, it is the case for example of some FDH species, but not the one used in this work, that have a tungsten electrical active site and do not need of cofactor. Other possibility is the use of a mediator that works transporting the electrons from the electrode to the enzyme; this is the case of the enzymes used in the present work. The FDH and the ADH would do the reaction also without the

potential apply, it through the sacrifice of the NADH cofactor, molecule that can gives the electrons and the protons needed to the reaction. In fact, in the introduction it has been mentioned that it is a bottle neck in the applications of the dehydrogenase enzymes, because the cofactor is an expensive reactive and many researches have been made to regenerate it. One option is the application of high over potentials. This electrochemical reduction is which should be happening when the enzyme reactions are observed in the cyclic voltammetries, i.e. the NADH is working as mediator. In this sense, it is very coherent to observe the preference of CF_{nt}+FDH_{Imm} for CO_2, while the CF_{nt}+ADH_{Imm} should correspond just to the HER because the substrates required to this enzyme are aldehydes, to be converted in alcohols, and it is known that one of characteristic of biocatalysts is their high selectivity. [35][36][37]

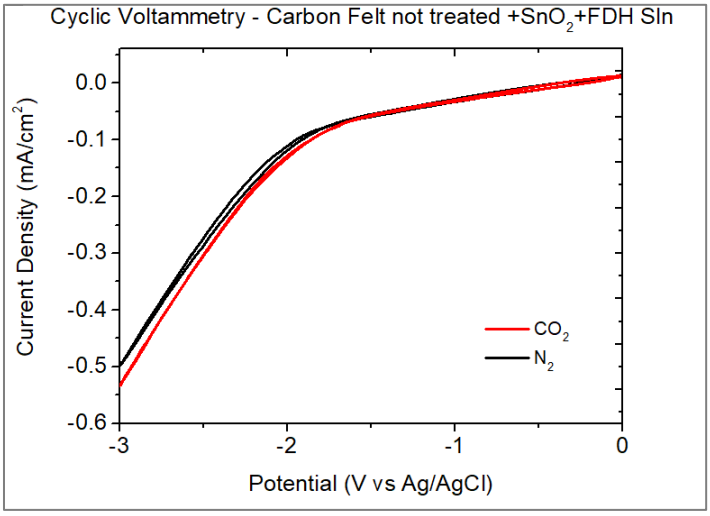

Figure 3.14. Last cycle of CVs on CF_{nt} + SnO_2 + FDH_{sln} in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}

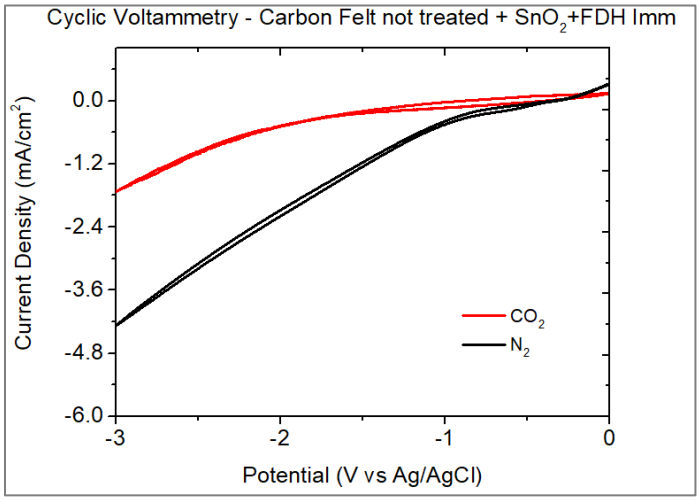

Figure 3.15. Last cycle of CVs on CF_{nt} + SnO_2 + FDH_{Imm} electrode in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}

The FDH and the SnO_2 are reported in literature to produce formate from CO_2 reduction, so it is interesting to observe if they can work in the same system, or even, if their can have a synergic behavior to improve their performance. This hybrid system has been tested by adding the protein in the electrolyte solution and also immobilizing it in the SnO_2 electrode, Figure 3.14 and Figure 3.15, respectively. There is a contrast behavior between both experiments, the CVs of CF_{nt} + SnO_2 + FDH_{Sln} experiment show lower currents than the electrocatalyst and the biocatalyst working separately. However, while in the CV curves of the CF_{nt} + SnO_2 it have not been seen a preferential activity in the presence of the CO_2, here it is observed. On the other way, the CF_{nt} + SnO_2 + FDH_{Imm} electrode in CO_2 saturated electrolyte presented current densities with magnitudes between the ones obtained for each individual catalyst, but higher currents in the nitrogen environment were obtained.

The low currents observed with the enzyme in solution, can be attributed to a lower interaction of the free enzyme with the electrode surface. If the enzymatic reduction with the cofactor mediation is occurring far away of the electrode, the cofactor regeneration will be more difficult; consequently, the reaction is not represented by the measured current density and, eventually, can be limited by the diminution of the NADH concentration. With respect to the bare SnO_2 electrocatalyst, the FDH in solution seems to have an inhibitory effect on the SnO_2 activity. A possible explanation could be that the enzyme, in some way, difficult the arrival of the CO_2 to the electrode surface. However, also the current density under N_2 flow was also significantly reduced. For the CF_{nt}+SnO_2+FDH_{Imm} electrode slightly higher current densities were observed in comparison to the CF_{nt} + SnO_2 electrode, but it should be verified with the products if actually, this behavior corresponds to an increase in the CO_2 reduction or just in the hydrogen evolution.

The second hybrid system (Cu + ADH) showed the highest current densities of all the CV tests. However, similar curves between both gasses have been obtained with slightly bigger current densities under N_2 saturation. In the first part of the scan it is possible to see a very defined redox couples, in N_2 atmosphere, and a more oxidative signal, with the CO_2. These signals could correspond to the signals observed in the CF_{nt}, the CF_{nt} + Cu and in the CF_{nt} + ADH. The capacitive effect has grown; like if certain kind of resistance is being generated after both, Cu deposition and enzyme immobilization methods used subsequentially. For example, an excess in Nafion could create a mass transfer barrier for the CO_2 difussioninto the electrode surface. Still, an important general result evidenced with the hybrid system is the increase in the current density in reference with the inorganic catalyst.

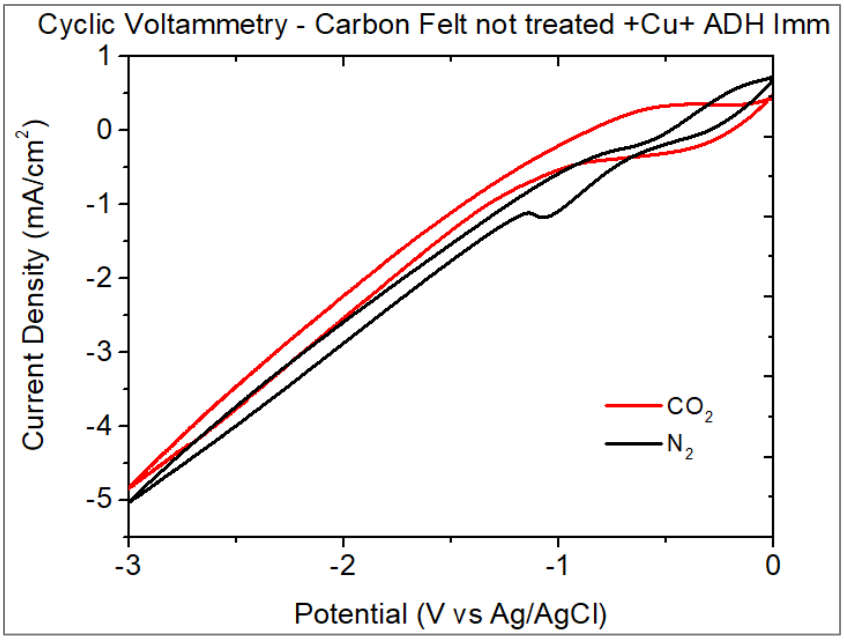

Figure 3.16. Last cycle of CVs on the CFnt+Cu+ADH_{Imm} in N_2 and CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}

- **Enzyme and Hybrid system Linear Sweep Voltammetries**

The LSV diagrams in Figure 3.17 summarize most of the results mentioned in the CVs tests, for each designed hybrid system. For the SnO_2 with FDH, the worst reduction activity was observed with the enzyme in solution system. The hybrid system with immobilized enzyme had an intermediate activity between the one of the inorganic catalyst and the one with the biocatalyst in the CF. The formate dehydrogenase immobilized presents the higher current densities. It should be remembered that the Cu and the ADH are not expected to work in the same way of SnO_2 with FDH, instead of looking for the production of the same compound in both kinds of catalysts, a sequential reaction was expected: first the CO_2 reduction to aldehydes should happen on the Cu surface, which acts as the substrate of the ADH that transform such aldehydes to alcohols. However, the results indicated that even if they have different mechanisms, it should be a common benefit in the use of both kind of catalyst in reference to the utilization of the classical electrocatalysts.

In the LSV it is easier to see the similar behavior between the curve of CF_{nt}+SnO_2+FDH_{Imm} and the inorganic cathode, CF_{nt}+SnO_2. After the reduction onset (around 1.5 V) the LSV comes apart a little, showing the possible contribution of the enzyme to the catalytic activity. The same behavior can be described for the Cu and the alcohol dehydrogenase catalysts but a bigger increment in the current density is observed in this hybrid system (see Figure 3.18).

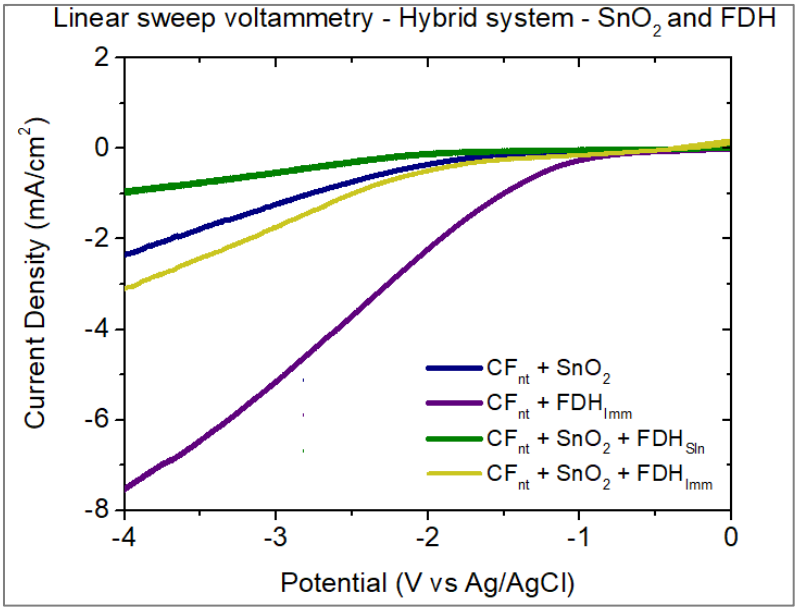

Figure 3.17. Linear scan voltammetry on 4 electrodes in CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at a scan rate of 30 mV s^{-1}.

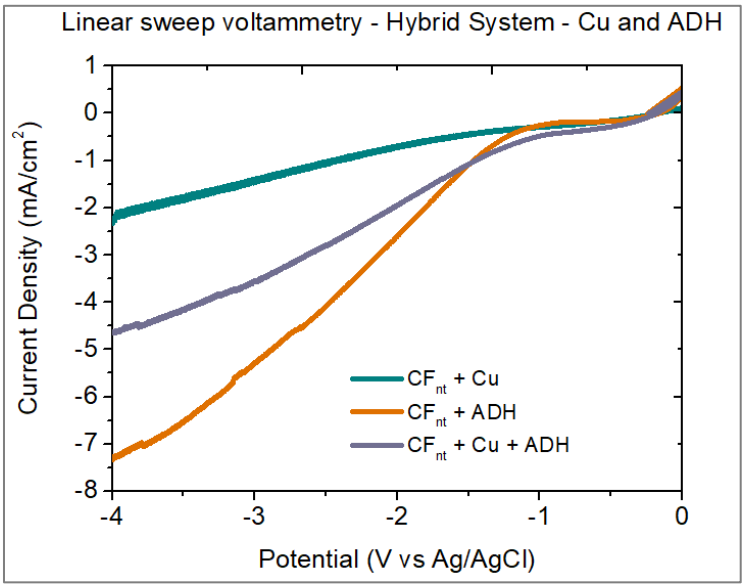

Figure 3.18. Linear scan voltametry on 3 electrodes in CO_2 saturated 0.1 M KHCO3 aqueous solutions at a scan rate of 30 mV s-

- **Enzyme and Hybrid system Chronoamperommetries**

Figure 3.19 shows the hydrogen production of the different samples. Also, as was explain previously, the degas part of the experiment were estimated to complete the hydrogen produced quantification in all experiments.

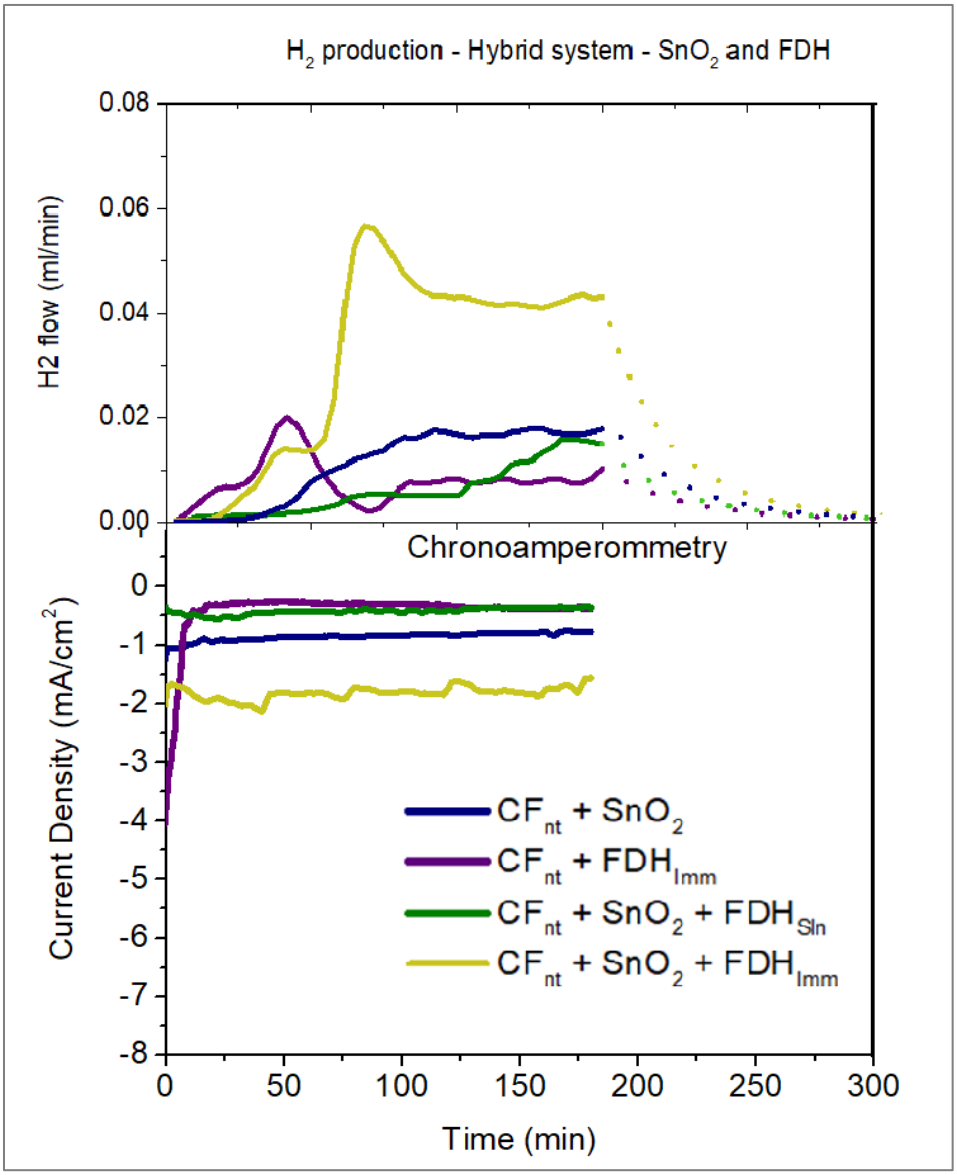

Figure 3.19. Hydrogen volumetric flow (upper grapgh) measured with a continuous gas chromatograph during the chronoamperommetries (lower graph) on 4 electrodes in CO_2 saturated 0.1 M $KHCO_3$ aqueous solutions at -3 V vs Ag/AgCl. The last part of H_2 flow diagrams (dot line) have been modeled instead of measured.

The CA for the CF_{nt} + FDH showed a lower steady state current density value than such expected by the LSV; it begins with a higher reducing current, but suffers a sharp drop of activity after a few minutes. This result is coherent with the enzyme expected behavior because at the beginning a higher concentration of cofactor is present in the solution, then, it is possible that the reaction is limited by the NADH regeneration. In this way, even if in the CV and the LSV of the electrode with the immobilized enzyme showed the better electrochemical activity, the higher steady state current densities were evidenced with the CF_{nt}+SnO_2+FDH_{Imm} samples, for which the CA current densities corresponds very good with the expected one observed from the LSV at the same applied potential. In general, the

CA curves evidence some unstable peaks, which could be attributed to the removal of produced gases (e.g. H_2) or to the unstable characteristic of the enzymes. Indeed, this test also has presented the larger hydrogen flow production, so the respective Faradaic efficiencies have been calculated and will be discussed below to quantify the real catalytic activity of the different electrodes. It should be also noted the similar behavior of the hydrogen curve of the electrode with only the enzyme and of the hybrid system.

As was anticipated the CF_{nt}+FDH_{Sln} experiment present a current density around -0.5 mA/cm^2. The H_2 production is also quiet low, but with the particularity that arrives to the steady state production of the CF_{nt}+SnO_2. This behavior was also seen for the SnO_2 deposited on the treated material. It was proposed that the treatment generates a catalyst distribution deeper in the carbon felt thickness, so the mass transfer problem limitations have been increased. Thus, a possibility is that enzyme in solution also can generate a kind of mass transfer problem.

The CA and H_2 production curves of the CF_{nt} + Cu, the CF_{nt} + ADH and the CF_{nt} + Cu + ADH electrodes are presented in Figure 3.20. The H_2 measurements for the CF_{nt} + Cu + ADH experiment is remarkable, around 75 minutes a maximum peak appeared, of more or less 0.12 ml/min H_2, then it rapidly decreased, and finally, at around 150 minutes the hydrogen reaction already tended to zero. As the current density remained almost constant, this phenomena can be an evidence of the sequential working of the Cu catalyst and the enzyme, where the first part corresponds to the work of copper, that produce in parallel hydrogen and some CO_2 reduced products (including provably some aldehydes) and the the aldehydes are reduced by the action of the enzyme. However, this hypothesis should be confirmed with products quantification and should be also proposed a logical mechanism for which the biocatalyst activity could inhibits the HER reaction.

The second Cu+ADH-based hybrid system differs from the SnO_2+FDH-based as soon as the magnitudes of current densities agreed with the information obtained from the LSVs curves. With the bare ADH enzyme electrode there is also an initial fast fall of current, but it stabilized at around -5 mA/cm^2, from where then it has slowly decreased up to about -4 mA/cm^2, which was the steady state current density reached by the Cu + ADH-hybrid electrode. Clearly, it is unlikely that the ADH would reduce the carbon dioxide, so the observed current is coherent with the high H_2 flow. Finally, the test with the ADH enzyme electrode was degassed until no hydrogen was detected by the GC, the results were used to determine the degassing model that was later used to estimate the hydrogen outgassing behavior with the other tests.

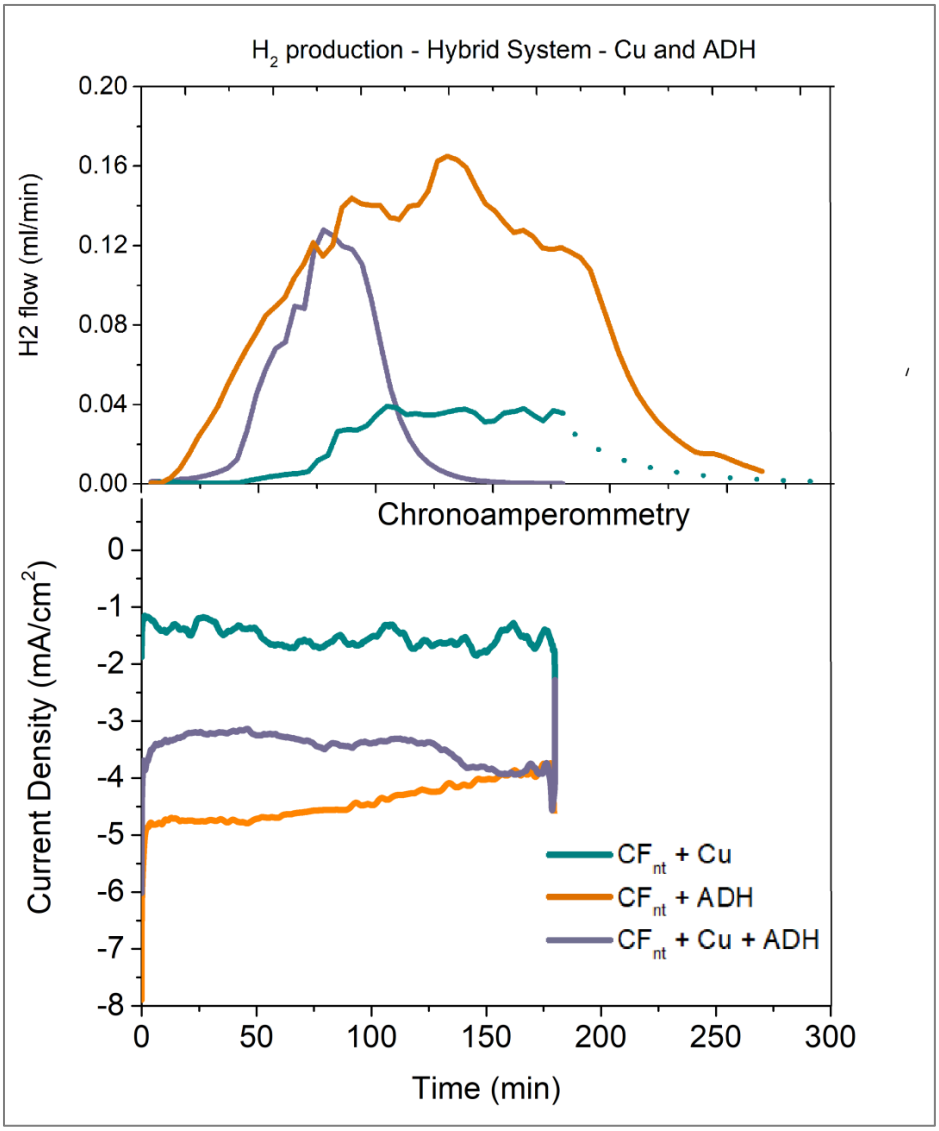

Figure 3.20. Hydrogen volumetric flow (upper grapgh) measured with a continuous gas chromatograph during the chronoamperommetries (lower graph) on 3 electrodes in CO_2 saturated 0.1 M KHCO3 aqueous solutions at -3 V vs Ag/AgCl. The last part of H_2 flow diagrams (dot line) have been modeled instead of measured.

- Faradaic efficiencies calculation

Only the micro-GC analyses and CA information are still not enough to know which catalysts present the better CO_2 reduction activity. The Table 3.6 summarizes the results for the electrochemical catalysts from both the micro-GC measurements and the other instrumental methods used to quantify the liquid products (HPLC and GC-MS), where formate, acetone and isopropanol were identified. From the gas flow measured by the micro-GC, a numerical integration has be done to quantify the total number of moles and the rate of production. Besides, with the current density data over the time from the potentiostat, again using a numerical integration, it was possible to calculate the total current density generated. Thus, the Faradaic efficiencies (FE) have been calculated,

Equation 1.4, and are reported in Figure 3.21. Due to the difference in the scale between the hydrogen FE and the FE for acetone and isopropanol, two axes have been used: left one for H_2 and the right one for the other two compounds.

Table 3.6. Summary of total current, products quantities, production rates and faradic efficiencies obtained with four catalysts during the chronoamperommetries on 4 electrodes in CO_2 saturated 0.1 M KHCO3 aqueous solutions at -3 V vs Ag/AgCl.

	CF_{nt}	**CF_t+SnO_2**	**CF_{nt}+SnO_2**	**CF_{nt}+Cu**
I_{total} (mA)	-7.789	-1.612	-2.690	-4.769
H_2 (µmol)	428.960	84.442	108.234	187.291
H_2 Production Rate (µmol/h*gcat)	953.244	0.169	2.268	0.187
$HCOO^-$ (µmol)	1.7799	3.9163	10.2238	2.3884
HCOO- Production Rate (µmol/h*gcat)	3.9552	78.4988	214.2005	57.6085
2-Propanol (mol)	0	0	0	0.013
2-Propanol Production Rate (µmol/h*gcat)	0	0	0	0.305
Acetone (mol)	0.059	0.102	0.431	0
Acetone Production Rate (µmol/h*gcat)	0.131	2.051	9.033	0

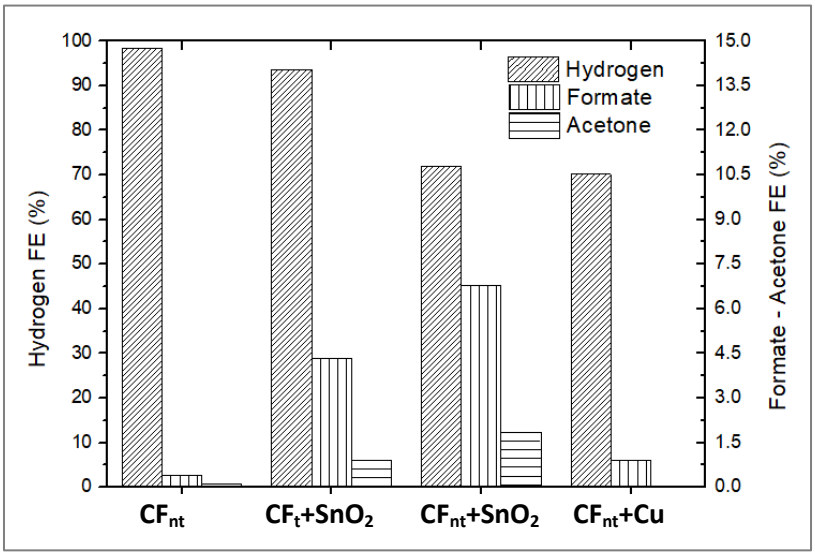

Figure 3.21. Faradic efficiencies for hydrogen (right axis), fomate and acetone (left axis) to four electrodes: Carbon felt not treated, carbon felt treated with SnO_2, carbon felt not treated with SnO_2 and carbon felt not treated with Cu.

Even if the CF_{nt} has the highest current densities, it is concluded that almost the whole reaction corresponds mainly to the HER, with a FE = 98.4 %. Nonetheless, the production of formate and acetone from this material (without any deposited catalyst) is notable, also if have efficiencies under 1%. It could be studied in the future the optimization of the support structure and the catalyst depositions to create a better catalytic system. Thus, the carbon dioxide reduction to formate was observed in all the proved inorganic systems, but, as expected, the SnO_2 electrodes produced five and seven times higher faradic efficiencies for formate than copper, in the CF_t and CF_{nt}, respectively.

It is interesting that the CF_{nt}+SnO_2 has a better activity for the CO_2 reduction than CF_t+SnO_2 electrode, which was already anticipated from the electrochemical curves analyzed. But the big difference is not seen in the Faradaic efficiencies for the quantified CO_2 reduction products, i.e. formate and acetone, for which the differences are of 2.5% and 0.3%, respectively. The much better behavior of the CF_{nt}+SnO_2 electrode can be observed because it consumes 22% less of electrons in the HER than the CF_t+SnO_2 one. In fact, the first one has missed around 20% of the total FE.

The CF_{nt} + Cu has a similar hydrogen reduction results (FE) in comparison with the CF_{nt} + SnO_2, but it should be pointed out that its total H_2 production is lower. For the CF_{nt} + Cu electrode the only quantified liquid product was formate and also there are missing near 30% of the FE for CO_2 reduction. In this sense, it should be said that the HPLC and GC-MS analysis methods are calibrated just to certain substances, so there are some peaks than have not been identified yet. Also, it is necessary take into account that a total of 60 ml of volume reaction have been required to the electrochemical cell, the recirculation and the external heating, what reduces the concentrations of the liquid products, so there can be carbon compounds in concentrations smaller of the instrumental detection limits. From literature, for example, is known that Cu can produce a wide list of possible carbon substances, until 16 products have found the majority with low concentrations. Finally, in future it also should be studied the final state of catalyst to study the final crystallinity and oxidation state of the cathode material.

The cathodes with enzymes had an additional problem for liquid products quantifications. The cofactor NADH has a very big signal in the HPLC detector (based on UV absorbance), so that it covers, for example, the formate peak. The method has been optimized to separate the peaks, but the new technique conditions also have produced bigger limitations in the detection and quantification limits. At this point it is sure that concentrations above 5 ppm can been quantified and the detection is lost in slightly lower concentrations. It indicates that the products missing to complete in some electrodes the total 100% of Faradaic efficiencies could be distributed in different compounds with lower concentrations. For example, after three hours of CA the cathodes CF_{nt} + SnO_2 + FDH_{Imm} and CF_{nt} + FDH have presented peaks of formate that should be, more or less, concentrations of 0.27 and 0.28 ppm, respectively 0.08 and 0.54% of FE. Also, the ADH electrode gave signals of formate of 0.54 ppm, for the first CA hour, and 0.94 ppm, for the third one (FE of 0.17%), which surely should be produced by the CF support and not by the enzyme. These values of faradic efficiencies are reported inside the total faradic efficiencies of carbon dioxide reduction products.

A similar situation occurred with GC-MS analysis; since the samples should be heated to

measure the volatile substances in head space volume of the vials, the NADH will be degraded generating multiple products that present peaks that interfere with the quantifications of the desired compounds. In this regard, as a future prospective, the development of suitable analytical and instrumentation methods to follows these hybrid systems is necessary. For the present work, the quantification have been done from the calculation of the hydrogen Faradaic efficiency, as an opposite indicator of the carbon dioxide reduction activity of the different electrocatalytic systems. The FE for all the test related to the first hybrid system (SnO_2+FDH) are shown in the Figure 3.22, while the Figure 3.23 shows the electrodes related to the second coupling (Cu+ADH). The missing faradic efficiencies are also reported. Taking these later values, and using in the opposite direction the Equation 1.4, an indicative quantity of an equivalent product (i.e. ethanol) was evaluated.

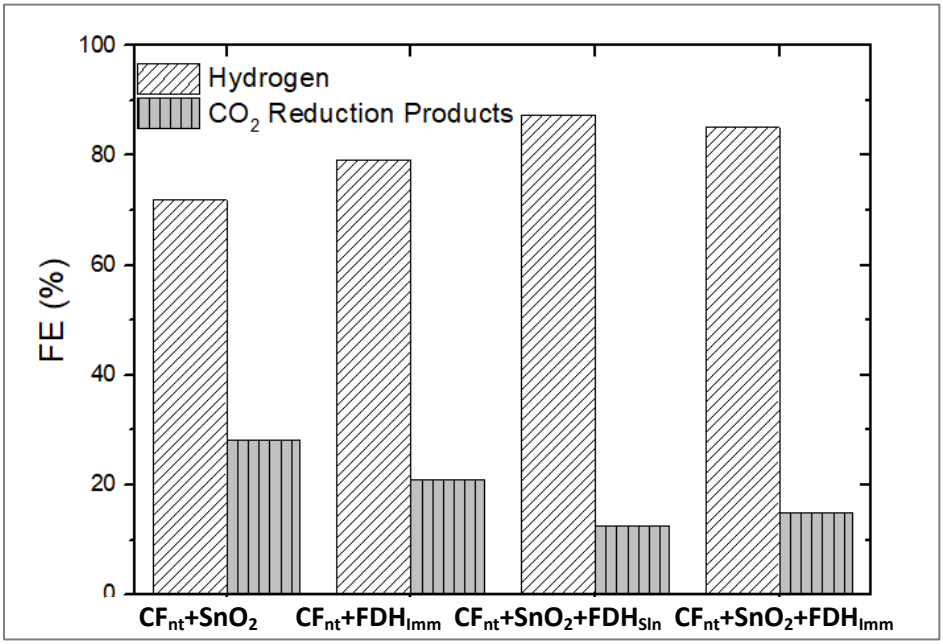

Figure 3.22. H_2 and CO_2 reduction products faradic efficiencies to four cathodes: carbon felt not treated with SnO_2, carbon felt not treated with FDH immobilized, carbon felt not treated with SnO_2 and FDH in solution and carbon felt not treated with SnO_2 and immobilized FDH.

In Figure 3.22, the electrode which present the lower FE for hydrogen and thus, by difference with the total 100%, the higher CO_2 reduction activity, is the inorganic SnO_2 catalyst, CF_{nt}+SnO_2. Previously, it have been hypothesized that the enzyme in solution, which has the worst catalytic performance, created a kind of interference for the CO_2 reduction in the electrode, this idea can be extended to the immobilized enzyme, which clearly have increased the currents, but at the same time the FE for HER was also raised. It can be related with the immobilization, for example the cathode with both, SnO_2 and FDH, should has a big quantity of Nafion, which also can act as a barrier for carbon dioxide

reduction. Other important parameter that should be evaluated in future is the influence of NADH concentration, this should be useful to improve the analytical and instrumentation methods, but even more important to know how the enzyme is working and if its activity can be fastened. Looking at the amounts of ethanol equivalent that could be produced, it can inferred that not all the missing FE (to achieve 100%) could be related to not quantified products. It is also possible that come electrons have been used for the reduction of the SnO_2 to metallic Sn in the catalyst surface, but this hypothesis could be confirmed only by further characterizations of the electrodes after the tests.

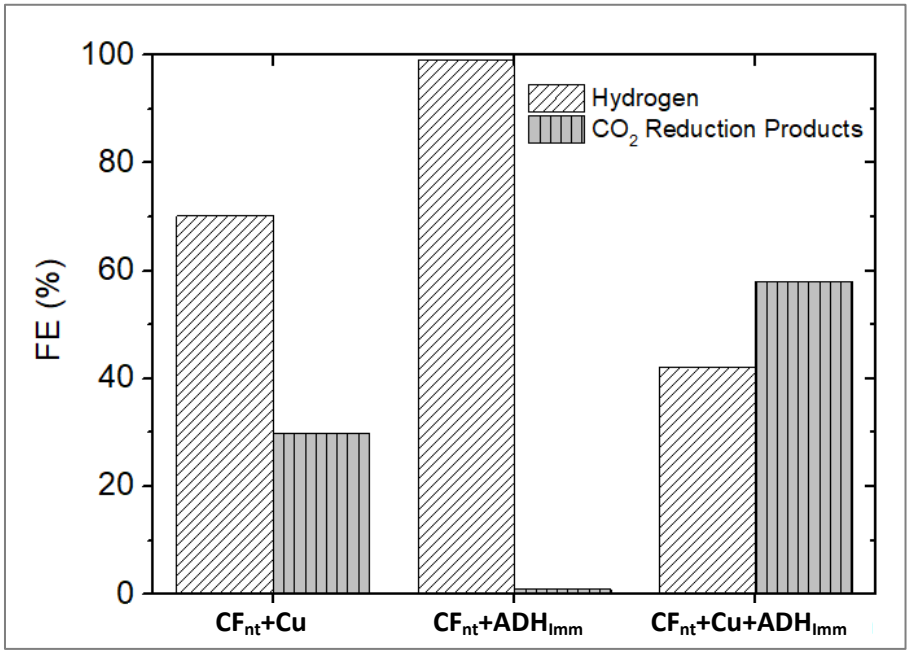

Figure 3.23. Hydrogen faradic efficiencies and missing percentages to three cathodes: carbon felt not treated with Cu, carbon felt not treated with ADH immobilized and carbon felt not treated with Cu and immobilized ADH. Indicative values of equivalence in ethanol concentration for unknown reactions are presented.

Finally, the second hybrid system showed very remarkable results. It should be remembered that with the electrode with the ADH enzyme have been evidenced the higher CA currents of all the here reported experiments, but as it has been expected, practically all the electrons transferred corresponded to the HER reaction (about 100% of FE for H_2). As the ADH do not produce in any way hydrogen, it means that the enzyme generates an important increase of current in the support, which is carrying out the reaction. The best results of all test can be attributed to the hybrid system of Cu and ADH, for which more than a half of the Faradaic efficiency should correspond to the CO_2 reduction reaction. Since, it was not possible to detect formate or alcohols from the HPLC measurements, it is possible that the liquid products can be distributed in a wide range of molecules in very low concentrations (< 5ppm). Also, it cannot be excluded that a part of the electrons also in this case were used for the reduction of some Cu-oxides (formed after the Cu thermal treatment) to metallic Cu.

4. Conclusions

Global warming is an important worldwide problem and the CO_2 emissions are one of the main concerns. Catalysts are being investigated to reduce the energy required to transform it to more complex molecules. Electrochemical reactions let to achieve transportation fuels, also have good controllability, efficiency and scalability, but in general have a low selectivity. In contrast, the most remarkable benefits of the use of biocatalysts, in addition to be environmentally friendly, are their high selectivity and specificity, but are more difficult to control, less flexible with the change of conditions and very instable.

This work focusses on the transformation of carbon dioxide in value-added chemicals with an electrocatalytic-enzyme hybrid system. First, tin oxide was coupled with the FDH, to evaluate the effect of combining these two types of catalyst on the production of formate. Then a sequential reaction was studied: the CO_2 reduction with a Cu-based catalyst to obtain aldehydes and a second step with the ADH to produce alcohols.

The specific activity of the alcohol dehydrogenase as free enzyme, in the oxidation reaction of ethanol at 30°C and pH 7.5, has been calculated to be 228.99 UI/mg_{enz}. Also for this enzyme, two immobilization processes have been tested. In the covalent bond technique the suitable time of immobilization has been identified to be 40 minutes and immobilization efficiencies have been calculated to be from 32% to 77%. Instead, in the Nafion entrapment process the entire enzyme amount is supposed to be fixed on the support. The activity of the immobilized enzyme was verified in both kind of immobilization with positive values of NADH concentrations, but repeatability problems have been seen for the covalent bond approach, where a specific value of activity has not been found. The activity of the immobilized enzyme by the entrapment method was very low, but this method allows a more homogeneous deposition and presents a good compatibility with the inorganic catalyst deposition. Hence, the entrapment enzyme immobilization method was chosen for the electrochemical tests. The SnO_2 and Cu catalysts deposition was done via spray coating and showed very good results, around 50% of efficiency for the SnO_2 and between 30% and 40% for the Cu.

Analytical and instrumental problems were evidence to quantify the liquid products, mainly due to the cofactor interference in the detector signal of the HPLC and due to its decomposition during the GC/MS analytic procedure. So the quantitative results of electrochemical tests were presented in terms of hydrogen production and Faradaic efficiency for H_2 evolution (FE_{H2}) and CO_2 reduction reaction (assumed to correspond to the difference between the FE_{H2} and 100%).

The electrochemical activity tests in an uncoated carbon felt (CF_{nt}) electrode that showed a very low FE of 1.6% to CO_2 reduction products, i.e. formate and acetone, and very high H_2 production selectivity. In all the inorganic catalysts the formate production has been found, in major quantity for CFnt+SnO_2, with FE around 7%. The worst performance was observed in the SnO_2 on the treated support; it has the higher H_2 faradic efficiency (93.6%) and the lower currents densities. Besides, two problems related with the thermal treatment have been hypothesized, the increase of the capacitor behavior and the mass transfer problems of the CO_2 to arrive to the catalyst. Both, CF_{nt}+SnO_2 and the CF_{nt}+Cu, have a Faradaic efficiency towards CO_2 reduction products around 30%, so the benefit seen in the copper is the quantity of current generated.

Important observations about the CVs and LSVs are the highest current showed by the enzymes. The FDH electrode showed a very clear preference for the CO_2 reduction, instead, for the ADH both CVs curves are very similar and make supposed that the capacitive properties of the electrode had increased, these characteristics are reflected in the CF_{nt}+Cu+ADH. The worst performance for the CO_2 reduction is evidenced with the FDH enzyme in solution (CF_{nt}+SnO_2+FDH_{Sln}), it generates the lower currents and the higher H_2 production. This can be explained because if the FDH reacts far from the electrode surface, it will be difficult to record the reaction rate with the electrochemical measurement, and it is possible that the NADH cofactor will not be easily regenerated. The high current decrease respect to the bare SnO_2 electrode can also be attributed to the block by the enzyme of the SnO_2 that is deposited in the electrode surface.

A common behavior of the hybrid systems is the increase of current respect to the inorganic catalysts, even if a lower efficiency for the CO_2 reduction was observed in CF_{nt}+SnO_2+FDH_{Imm}, more CO_2 reduction products in comparison to CF_{nt}+SnO_2 and CF_{nt}+FDH_{Imm} were obtained because of the higher current densities. It is a good indicator and maybe working on the enzyme deposition or operative conditions the hybrid electrochemical system can be further improved.

The highest currents were obtained with CF_{nt}+ADH_{Imm} and CF_{nt}+Cu+ADH_{Imm}. The ADH cannot react in any way with CO_2 and it was demonstrated by the H_2 Faradaic efficiency of 99.13%. On contrary, the FE_{H2} for the CF_{nt}+Cu+ADH_{Imm} is the lowest, indicating a better CO_2 reduction performance. This experiment also has presented a very interesting H_2 production profile, where H_2 increased up to a maximum and then has a slow decreased to zero in 150 minutes. It could represent the sequential desired reactions: first the Cu works with parallel production of H_2 and CO_2 reduction products, like aldehydes, which are then reduced by the ADH enzyme to produce alcohols.

5. Future Work

From the results obtained from this thesis work it comes out that researches in the quantifications methods are required for future improvements of the efficiency of the hybrid enzymatic-inorganic catalytic systems. To improve the hybrid electrochemical system, possible studies in the future may focus on the effect of the NADH concentration, the distribution and amounts of both catalysts, and the effect of the presence of Nafion in the electrode to avoid its effect as a possible barrier to the mass transfer of carbon dioxide. Finally, further investigations are necessary in order to overcome some critical issues related to analytical measures of liquid products and with the understanding of the reaction mechanism to enhance the CO2 reduction reaction and inhibit the HER

6. Bibliography

[1] United Nations Sustainable Development, "Climate Change - United Nations Sustainable Development," 2016. [Online]. Available: http://www.un.org/sustainabledevelopment/climate-change-2/.

[2] Y. Zheng *et al.*, "Energy related CO2 conversion and utilization: Advanced materials/nanomaterials, reaction mechanisms and technologies," *Nano Energy*, vol. 40, no. April, pp. 512–539, 2017.

[3] A. Alissandratos and C. J. Easton, "Biocatalysis for the application of CO2 as a chemical feedstock," *Beilstein J. Org. Chem.*, vol. 11, pp. 2370–2387, 2015.

[4] F. Marpani, M. Pinelo, and A. S. Meyer, "Enzymatic conversion of CO2 to CH3OH via reverse dehydrogenase cascade biocatalysis: Quantitative comparison of efficiencies of immobilized enzyme systems," *Biochem. Eng. J.*, vol. 127, pp. 217–228, 2017.

[5] J. Luo, A. S. Meyer, R. V. Mateiu, and M. Pinelo, "Cascade catalysis in membranes with enzyme immobilization for multi-enzymatic conversion of CO<inf>2</inf> to methanol," *N. Biotechnol.*, vol. 32, no. 3, pp. 319–327, 2015.

[6] V. J. G. Voet Donald, "Rates of Enzymatic Reactions," in *Biochemistry*, 4th ed., 2010, pp. 482–505.

[7] R. Barin, D. Biria, S. Rashid-nadimi, and M. Ali, "Enzymatic CO 2 reduction to formate by formate dehydrogenase from Candida boidinii coupling with direct electrochemical regeneration of NADH," vol. 28, no. October, pp. 117–125, 2018.

[8] N. H. Ibáñez, "Exploration of novel materials in (bio) electrocatalysis : sensing in complex media and biocathodes for the CO2 reduction Naiara Hernández Ibáñez Facultad de Ciencias Exploration of novel materials in (bio) electrocatalysis : sensing in complex media," Universidad de Alicante, 2018.

[9] A. Illanes, *Enzyme biocatalysis: Principles and applications*. 2008.

[10] S. Bajracharya, S. Srikanth, G. Mohanakrishna, and R. Zacharia, "Biotransformation of carbon dioxide in bioelectrochemical systems : State of the art and future prospects," *J. Power Sources*, vol. 356, pp. 256–273, 2017.

[11] H. W. D. Wang, Yamen; He, Da; Chen, "Catalysts in electro-, photo and photoelectrocatalytic CO2 reduction reactions," *J. Photochem. Photobiol. C Photochem. Rev.*, pp. 1–33, 2019.

[12] Y. Chen and M. W. Kanan, "Tin oxide dependence of the CO 2 reduction efficiency on tin electrodes and enhanced activity for tin/tin oxide thin-film catalysts," *J. Am. Chem. Soc.*, vol. 134, no. 4, pp. 1986–1989, 2012.

[13] K. Bejtka *et al.*, "Chainlike Mesoporous SnO2 as a Well-Performing Catalyst for

Electrochemical CO2 Reduction," *ACS Appl. Energy Mater.*, vol. 2, no. 5, pp. 3081–3091, 2019.

[14] Q. Lu, J. Rosen, and F. Jiao, "Nanostructured metallic electrocatalysts for carbon dioxide reduction," *ChemCatChem*, vol. 7, no. 1, pp. 38–47, 2015.

[15] K. P. Kuhl, E. R. Cave, D. N. Abram, and T. F. Jaramillo, "New insights into the electrochemical reduction of carbon dioxide on metallic copper surfaces," *Energy Environ. Sci.*, vol. 5, no. 5, pp. 7050–7059, 2012.

[16] S. Kim, M. K. Kim, S. H. Lee, S. Yoon, and K. D. Jung, "Conversion of CO2 to formate in an electroenzymatic cell using Candida boidinii formate dehydrogenase," *J. Mol. Catal. B Enzym.*, vol. 102, pp. 9–15, 2014.

[17] T. X. Huong Le, M. Bechelany, and M. Cretin, "Carbon felt based-electrodes for energy and environmental applications: A review," *Carbon N. Y.*, vol. 122, pp. 564–591, 2017.

[18] J. Ashenhurst, "Opening of Epoxides With Acid — Master Organic Chemistry." [Online]. Available: http://www.masterorganicchemistry.com/2015/02/02/opening-of-epoxides-with-acid/.

[19] J. Ashenhurst, "Reagent Friday_ Sodium Periodate – Master Organic Chemistry." [Online]. Available: https://www.masterorganicchemistry.com/2011/10/21/reagent-friday-sodium-periodate/.

[20] G. B. Damas *et al.*, "On the Mechanism of Carbon Dioxide Reduction on Sn-Based Electrodes : Insights into the Role of Oxide Surfaces," *Catalysts*, vol. 9, 2019.

[21] S. Zhao *et al.*, "Advances in Sn-Based Catalysts for Electrochemical CO2 Reduction," *Nano-Micro Lett.*, vol. 11, no. 1, 2019.

[22] A. Eilert, F. S. Roberts, D. Friebel, and A. Nilsson, "Formation of Copper Catalysts for CO2 Reduction with High Ethylene/Methane Product Ratio Investigated with in Situ X-ray Absorption Spectroscopy," *J. Phys. Chem. Lett.*, vol. 7, no. 8, pp. 1466–1470, 2016.

[23] International Energy Agency -IEA, "Global Energy and CO2 Status Report 2018," *Iea*. p. 29, 2019.

[24] Scripps Institution of Oceanography, "The Keeling Curve | A daily record of atmospheric carbon dioxide," *Scripps Institution of Oceanography*. 2019.

[25] M. B. Moran, Michael J; Shapiro, Howard N; Boettner, Daisie D; Bailey, *Fundamentals of engineering thermodynamics*, 7th ed. 2010.

[26] N. R. Mohamad, N. H. C. Marzuki, N. A. Buang, F. Huyop, and R. A. Wahab, "An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes," *Biotechnol. Biotechnol. Equip.*, vol. 29, no. 2, pp. 205–220, 2015.

[27] H. Jakubowski, “Biochemistry Online: Table of Contents,” *Chemistry Online: An Approach Based on Chemical Logic*, 2016. [Online]. Available: https://employees.csbsju.edu/hjakubowski/classes/ch331/bcintro/list_of_figures.htm.

[28] S. Liu and S. Huang, “Size effects and active sites of Cu nanoparticle catalysts for CO 2 electroreduction,” *Appl. Surf. Sci.*, vol. 475, no. December 2018, pp. 20–27, 2019.

[29] B. Zhang and J. Zhang, “Rational design of Cu-based electrocatalysts for electrochemical reduction of carbon dioxide,” *J. Energy Chem.*, vol. 26, no. 6, pp. 1050–1066, 2017.

[30] M. Behrens, “Heterogeneous Catalysis of CO2 Conversion to Methanol on Copper Surfaces,” *Angew. Chemie - Int. Ed.*, vol. 53, no. 45, pp. 12022–12024, 2014.

[31] S. Schlager *et al.*, “Biocatalytic and Bioelectrocatalytic Approaches for the Reduction of Carbon Dioxide using Enzymes,” *Energy Technol.*, vol. 5, no. 6, pp. 812–821, 2017.

[32] Y. J. Ma, J. L. Wang, and X. P. Cai, “The Effect of Electrolyte on Surface Composite and Microstructure of Carbon Fiber by Electrochemical Treatment,” *Int. J. Electrochem. Sci*, vol. 8, pp. 2806–2815, 2013.

[33] L. Eifert, R. Banerjee, and Z. Jusys, “Characterization of Carbon Felt Electrodes for Vanadium Redox Flow Batteries : Impact of Treatment Methods,” *J. Electrochem. Soc.*, vol. 165, no. 11, 2018.

[34] R. Díaz, I. Díez-pérez, P. Gorostiza, F. Sanz, and J. R. Morante, “An Electrochemical Study of Tin Oxide Thin Film in Borate Buffer Solutions,” *J. Braz. Chem. Soc*, vol. 14, no. 4, pp. 523–529, 2003.

[35] V. Flexer and N. Brun, “Fundamentals of Enzymatic Electrochemical Systems,” in *Functional Electrodes for Enzymatic and Microbial Electrochemical Systems*, WORLD SCIENTIFIC (EUROPE), 2017, pp. 3–50.

[36] F. A. Armstrong and J. Hirst, “Reversibility and efficiency in electrocatalytic energy conversion and lessons from enzymes,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 108, no. 34, pp. 14049–14051, 2011.

[37] A. R. Pereira, G. C. Sedenho, J. C. P. de Souza, and F. N. Crespilho, “Advances in enzyme bioelectrochemistry,” *An. Acad. Bras. Cienc.*, vol. 90, no. 1, pp. 825–857, 2018.