JOHANNES KEPLER UNIVERSITÄT LINZ | JKU

TNF

Technisch-Naturwissenschaftliche Fakultät

Enzymatic Reduction of Carbon Dioxide to Formate

Bachelor Thesis in Biological Chemistry

Daniela Hiemetsberger

Supervisor: DI Stefanie Schlager
o. Univ. Prof. Dr. Mag. Niyazi Serdar Sariciftci

Institute of Physical Chemistry, JKU

Bachelor thesis

Hiemetsberger D., 2013: Enzymatic Reduction of Carbon Dioxide to Formate, BSc. Thesis in English, 41 p., Institute of Physical Chemistry, Johannes Kepler Universität, Linz, Austria

Abstract

This thesis presents the immobilization of the enzyme formate dehydrogenase in alginate based matrices for sustainable, repeatable CO_2 reduction. Different alginate containing gels, modified with PEDOT:PSS are investigated for efficient CO_2 conversion. The first method presented is limited due to necessity of the co-enzyme nicotinamide adenine dinucleotide (NADH). The second approach offers the opportunity for electrochemical application of the enzyme containing systems and therefore to substitute NADH as electron provider. NADH is replaced by direct electrochemical reduction of immobilized formate dehydrogenase enzyme for CO_2 conversion to formate using modified electrochemical electrodes. Production of formate is shown for the enzymatic CO_2 reduction in electrochemical and non-electrochemical experiments. Products were analysed in ion chromatography. Electrochemical measurements were performed in a one compartment cell using alginate covered Pt and Carbon felt as working electrode. Cyclic voltammograms were recorded for electrochemical characterisation. Results from CO_2 saturated samples are compared to N_2 purged setups to proof product generation from CO_2 reduction.

Abstrakt

Diese Arbeit präsentiert die Immobilisierung des Enzyms Formatdehydrogenase in Alginat basierten Matrizen für eine nachhaltige, reproduzierbare CO_2 Reduktion. Verschiedene Gele, welche mit PEDOT:PSS modifiziert wurden, werden für eine effiziente CO_2 Umwandlung untersucht. Das erste vorgestellte Verfahren ist aufgrund der Notwendigkeit des Coenzyms Nicotinamidadenindinukleotid (NADH) begrenzt. Der zweite Ansatz bietet die Möglichkeit für die elektrochemische Anwendung der enzymhaltigen Systeme und damit NADH als Elektronenquelle zu ersetzen. NADH wird durch direkte elektrochemische Reduktion von immobilisierter Formatdehydrogenase mit modifizierten elektrochemischen Elektroden ersetzt. Die Produktion von Formiat für die enzymatische CO_2 Reduktion in elektrochemischen und nicht-elektrochemischen Experimenten wird gezeigt. Produkte wurden mit Ionenchromatographie analysiert. Elektrochemische Messungen wurden in einer Einkammerzelle mit Pt -und Carbonfilz als Arbeitselektroden durchgeführt. Zyklische Voltammogramme wurden für die elektrochemische Charakterisierung aufgezeichnet. Um die Produktion von CO_2 zu überprüfen wurden Proben aus den CO_2 gesättigten Setups und N_2 gespülten Setups verglichen.

Acknowledgements

I am grateful to DI Stefanie Schlager for her excellent guidance in the laboratory. I also want to thank her for the supervision and support of my bachelor thesis.
Many thanks to MSc Dogukan H. Apaydin who always answered upcoming questions and helped me handling the analytical instruments.
I also would like to express my thanks to o. Univ. Prof. Dr. Mag. Niyazi Serdar Sariciftci for giving me the opportunity to work at his institute.

Table of Contents

1. Introduction

1.1 Carbon Dioxide as Greenhouse Gas.....1

1.2 Carbon Capture and Storage.....3

1.3 Carbon Capture and Utilization.....4

1.4 CO_2 conversion with enzymes.....6

1.5 Enzymes as catalysts.....7

1.6 Immobilisation of enzymes.....11

2. Experimental Part

2.1 Non-electrochemical experiments.....13

2.1.1 Preparation of Alginate-Silicate Beads.....13

2.1.2 CO_2 reduction experiments (Alginate-Silicate Beads) 14

2.1.3 Preparation of Alginate Beads containing PEDOT:PSS.....14

2.1.4 CO_2 reduction experiment (Alginate-Silicate Beads containing PEDOT:PSS)...15

2.2 Electrochemical experiments.....16

2.2.1 Preparation of coated electrodes.....17

3. Results and Discussion

3.1. Non-electrochemical experiments.....18

3.1.1 Alginate-Silicate Beads.....18

3.1.2 Alginate-PEDOT:PSS Beads.....20

3.2. Electrochemical Experiments.....22

3.2.1. Alginate Carbon Felt Electrode.....22

3.2.2. PEDOT:PSS Alginate Carbon Felt Electrode.....28

4. Conclusion 34

References.....35

1. Introduction

1.1 Carbon Dioxide as Greenhouse Gas

Nowadays, global warming is the major environmental issue worldwide[1]. This problem is strongly correlated with carbon dioxide which accounts as main anthropogenic greenhouse gas. The CO_2 emission levels are increasing rapidly due to combustion of fossil fuels by industry and transport. Further, deforestation interferes with photosynthesis and decreases therefore natural CO_2 regeneration.[2] The world economies depend on fossil fuels as energy source, which leads to an increase of CO_2 emissions.[3] Currently fossil fuel fired power plants are responsible for 80% of total energy production worldwide, thus they are the largest source of CO_2 emissions, accounting for roughly 40% of total CO_2 emissions.[4]

There is an urgent need to reduce atmospheric CO_2 emissions as natural occurring photosynthesis is not longer able to convert all the masses of CO_2 which are produced throughout the world.

The Keeling curve illustrates the rising atmospheric CO_2 content in the atmosphere of the past 60 years. Continuous CO_2 measurements from Mauna Loa, Hawaii (blue) and at the South Pole (red) predict both a constant increase of the atmospheric CO_2 level. Recently the amount of CO_2 in the earth's atmosphere was determined at 393 ppm by the Mauna Loa Observatory (see Figure 1).[5]

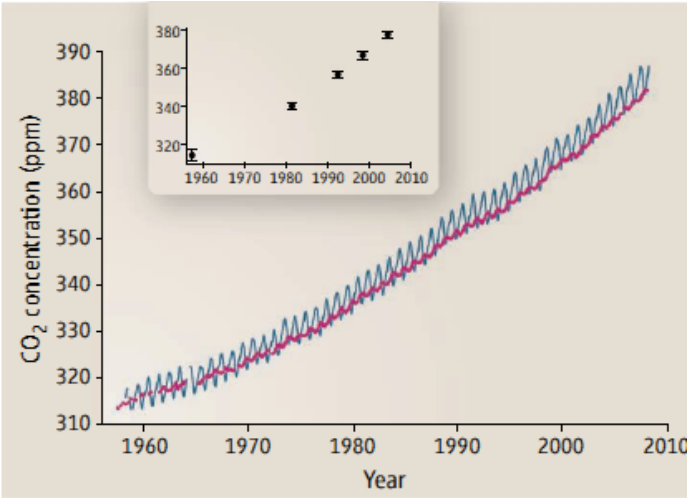


Figure 1: Constant increase of the CO_2 levels from records from Mauna Loa, Hawaii (blue) and at the South Pole (red).[5]

Further, Figure 2 shows records of the Vostok ice core. The graph demonstrates that temperature is parallel ongoing with increase of atmospheric CO_2. Although the atmospheric CO_2 content was following fluctuation over time it is obvious that now the highest level ever has been reached and there is no decrease of CO_2 content expected, as it was observed in former times. However, it is alarming that the CO_2 concentration was increasing rapidly in the last few years and it is proven that this correlates to the global warming that is proven by melting glaciers and significant changes in nature and environment.[6]

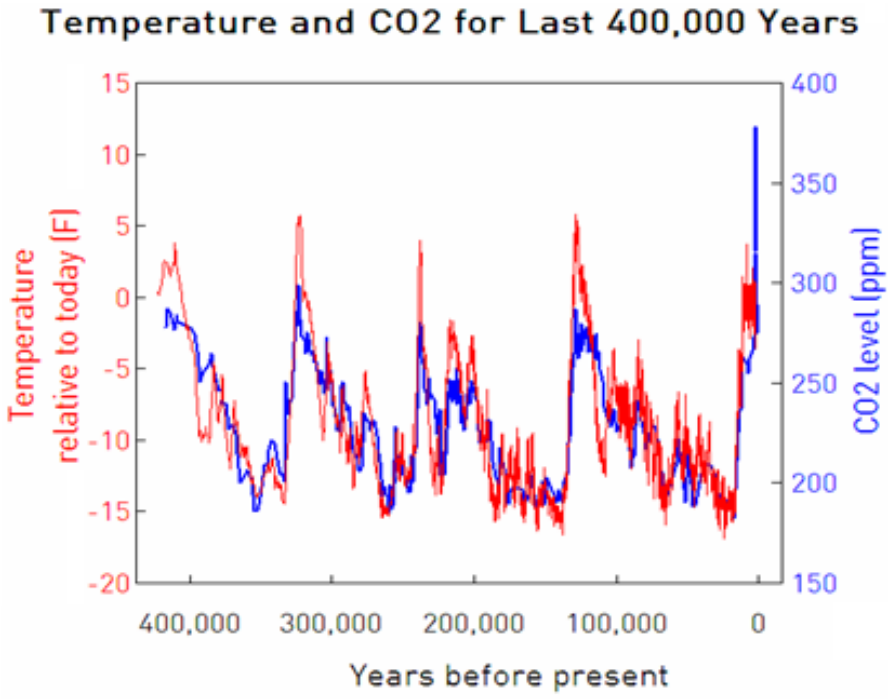


Figure 2: Records of the Vostok ice core demonstrate the change in temperature and CO_2 level in the last 400 000 years.[6]

Recycling of CO_2 to useful fuels and organic molecules offers great potential to decrease greenhouse gas emissions. Further CO_2 offers high potential as carbon source and represents therefore a source for sustainable energy.

There are several approaches to decrease atmospheric CO_2. In this work we present the chemical and electrochemical reduction of CO_2 using enzymes as catalysts which will be discussed in detail in section 1.4 (CO_2 conversion by enzymes) and section 1.5 (Enyzmes as catalysts).

1.2 Carbon Capture and Storage

One approach of decreasing atmospheric CO_2 is Carbon Capture and Storage (CCS). CCS is a combination of technologies to stabilize the CO_2 concentration in the atmosphere while maintaining carbon as the main energy source.

The CCS methodologies consist of three major steps: First the capture of CO_2 from large point sources, such as power plants and cement manufacturing facilities. In the next step this gas mixture is liquefied and transported by pipeline or ship to the storage site. In the last step CO_2 is injected into the storage site. The CO_2 storage options comprise geological storage, ocean storage and mineralization.[3]

A commercially attractive way of CO_2 storage is the injection of CO_2 into oil reservoirs to enhance oil recovery (EOR).[2] Further, EOR storage is also possible in deep unmineable coal seams, with enhanced coal bed methane recovery (ECBMR) offsetting some of the costs of storage. [7]

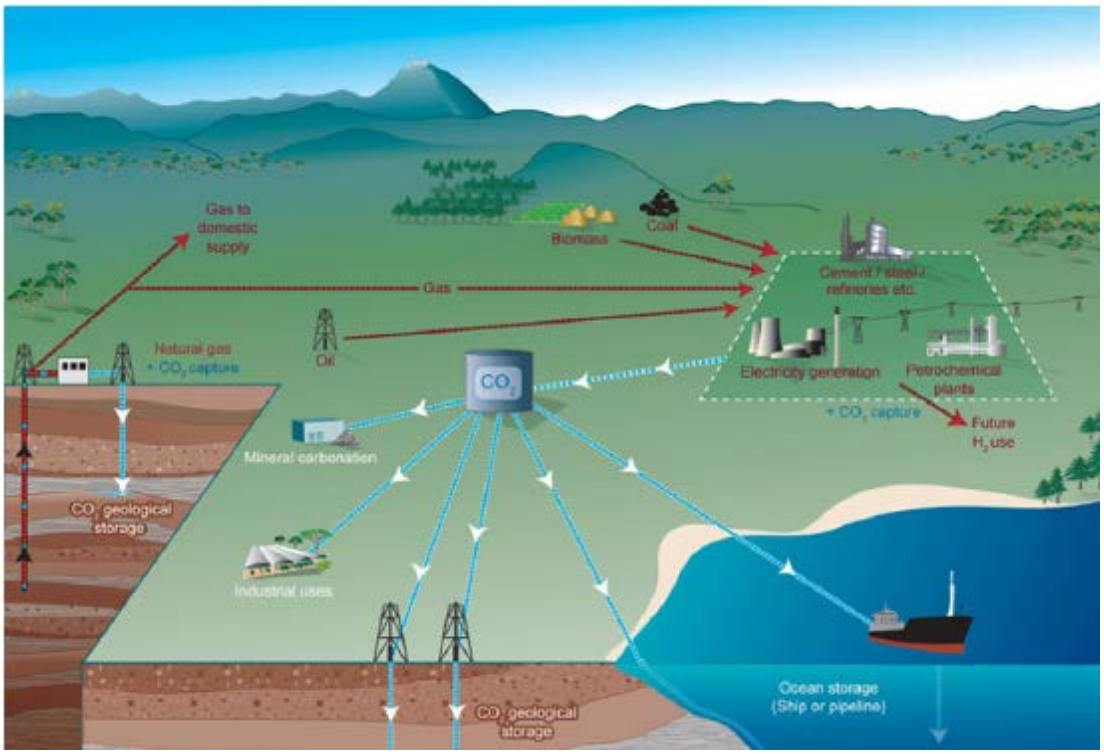


Figure 3: Schematic view of a possible Carbon Capture and Storage chain (IPCC, 2005).[3]

In comparison to a power plant without CCS, a modern conventional power plant with CCS could reduce CO_2 emissions into the atmosphere by approximately 80-90%.[2]

However, the CCS concept is connected to high costs. The costs for a plant with CO_2 capture are higher due to increased investment costs, reduced plant efficiency, and increased maintenance costs, as well as the costs for CO_2 capture, transport and storage. In addition, the environmental aspects also have to be considered as well as the risk of any leakage to the surface.[8]

1.3 Carbon Capture and Utilization

Apart from Carbon Capture and Storage, Carbon Capture and Utilization (CCU) offers favourable possibilities of addressing the CO_2 problem. The aim of CCU is to convert CO_2 to fuel and other value-added products and simultaneously reducing atmospheric CO_2. The products obtained could supplement or replace chemical feedstocks in chemical, pharmaceutical and polymer industries. Despite these approaches at the moment less than 1% of global anthropogenic CO_2 generated is utilized to useful products. The residual CO_2 is released to the atmosphere due to lack of economical technologies.1

CO_2 is kinetically and thermodynamically stable, thus CO_2 conversion reactions are endothermic, which is a major difficulty in establishing industrial processes as any reaction converting CO_2 requires an energy input. Therefore, it would be reasonable to think about renewable and especially excess energies as energy sources for CO_2 conversion.2

Several approaches of CO_2 utilization were presented by Aresta[9,10] and George Olah[11]. They investigated mainly the reduction of CO_2 to several organic products and fuels like methanol. Equation 1 depicts the standard redox potentials vs. normal hydrogen electrode for the reduction of CO_2 to different products in aqueous solution at pH 7, 25°C and 1 atm.

$CO_2 + e^- \rightarrow CO^{*-}$	$E^0 = -1.90$ V
$CO_2 + 2e^- + 2H^+ \rightarrow CO + H_2O$	$E^0 = -0.53$ V
$CO_2 + 2e^- + 2H^+ \rightarrow HCOOH$	$E^0 = -0.61$ V
$CO_2 + 4e^- + 4H^+ \rightarrow H_2CO + H_2O$	$E^0 = -0.48$ V
$CO_2 + 6e^- + 6H^+ \rightarrow CH_3OH + H_2O$	$E^0 = -0.38$ V
$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O$	$E^0 = -0.24$ V

Equation 1: Standard redox potentials for CO_2 reduction at pH 7 in aqueous solution vs. a normal hydrogen electrode at 25 °C and1 atm.

However, CO_2 is a stable molecule and indeed those potentials are higher in reality. Most reactions require therefore high overpotentials for the reduction of CO_2. To overcome the thermodynamic barriers of CO_2 reduction catalysts are necessary. They are used to lower the overpotential by stabilizing the intermediate transition states between the linear CO_2 molecule and the intended product.[12]

In general, catalysis can be divided into two main application techniques: homogeneous and heterogeneous. Homogeneous catalysts are in the same phase as the reactants in contrast to heterogeneous catalysts which are in a different phase. The major advantage of heterogeneous catalysts is the opportunity for the reusability of the catalyst and easier separation of catalyst and product.

In the 1980's electrochemical and photochemical approaches for the reduction of CO_2 to CO have been investigated by Lehn et al. The [fac-Re(2,2'-bipyridine)$(CO)_3$Cl] complex was used as an efficient homogeneous metal-organic catalyst for the electrocatalytic reduction of CO_2.[13] It was applied in solution as well as with polypyrrole electrodes as demonstrated by the group of Cosnier et al.[14] Later, approaches of using such metal organic catalyst for the photoelectrochemical and electrochemical CO_2 reduction were done by the group of Kubiak.[15]

Further pyridine was used as homogenous catalyst for CO_2 reduction by the group of Bocarsly. They investigated pyridine and its substituted derivatives as organic catalysts in the electrocatalysis of CO_2 redction to various products, such as formic acid, formaldehyde and methanol, operating at low reaction overpotentials and yielding faradaic efficiencies of up to 22% for methanol production.[16]

Besides metal-organic complexes and organic molecules recent research also focuses on biological systems as it was demonstrated by Li et al. The idea was to apply litoautrotrophic microorganisms for the electrochemical production of formate from CO_2 and H_2O and its conversion to higher alcohols.[17] In a different approach Jiang et al. present the generation of methane by methanogenous bacteria immobilized on carbon felt electrodes.[18]

This work focuses on another biocatalytic approach of CO_2 reduction, which is the application of enzymes and will be discussed in the following sections.

1.4 CO_2 conversion with enzymes

Enzymes depict the ability of catalysing CO_2 reduction at mild reaction conditions and to high yields. Further, biocatalysts possess favourable properties such as biodegradability and selectivity.

Already in 1976 the group of Ruschig et al. presented the reduction of CO_2 to formate with formate dehydrogenase and the co-enzyme NADH.[19] Later, Kuwabata et al. show the electrochemical reduction of CO_2 using dehydrogenases as well but substituting the co-enzyme NADH by chemical mediators.[20] In another approach Aresta et al. used carboxylase enzymes for the synthesis of benzoic acid from phenol and CO_2.[21] Obert and Dave presented further the use of dehydrogenase enzymes immobilized in silica sol gel for CO_2 conversion to methanol.[22] Adressing the substitution of NADH as well, Reda et al. showed the adsorption of an electroactive enzyme on electrode surfaces to catalyze the efficient reduction of CO_2 to formate, mediated by methylviologen. The reaction requires only small overpotentials and occurs under thoroughly mild conditions.[23]

In this work we concentrate on the formate dehydrogenase enzyme to proceed the $2e^-$ CO_2 reduction to formate. In the human body enzymes are responsible for the three step alcohol oxidation to CO_2. For CO_2 reduction we try to mime this reaction in the counter direction to reduce CO_2 to formate using dehydrogenase enzymes. Dehydrogenases are enzymes that transfer two hydrogen atoms from a reduced substrate to an electron acceptor. For this reaction dehydrogenases require a so called coenzyme which will function as the electron acceptor.[24]

In general, to reduce CO_2 to methanol three enzymes, formate dehydrogenase ($F_{ate}DH$), formaldehyde dehydrogenase ($F_{ald}DH$) and alcohol dehydrogenase (ADH), are required.[25] In addition NADH is used as sacrificial coenzyme for the hydrogen and electron transfer. This means that for each reduction step one NADH molecule is irreversibly oxidized to NAD^+ as depicted in Figure 4.

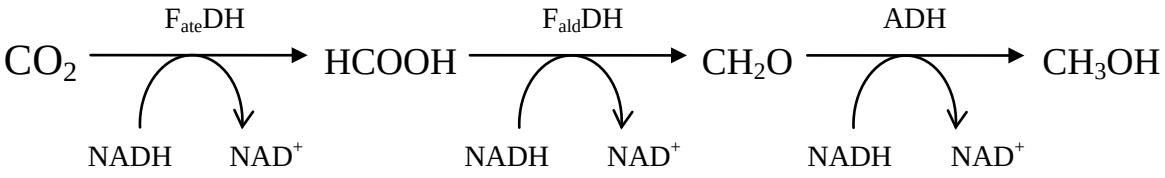


Figure 4: Reaction cascade of the 3-step enzymatic reduction of CO_2 to methanol with formate dehydrogenase, formaldehyde dehydrogenase and alcohol dehydrogenase and NADH as co-enzyme.

NADH regeneration, however, requires high energy inputs and increases therefore costs for enzymatic CO_2 reduction. In a new approach NADH can be substituted by direct electron injection from an electrochemical system as shown in Figure 5. Similar approaches were already done by Kuwabata and Reda.[20,23]

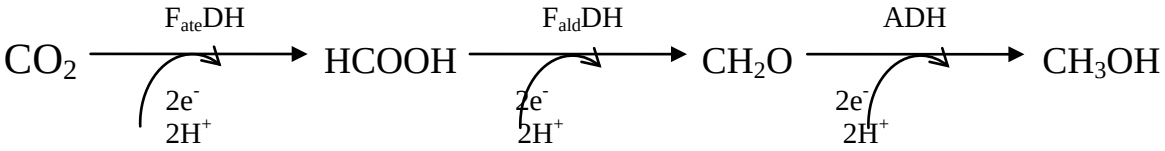


Figure 5: Reaction cascade of the 3-step reduction of CO_2 to methanol with formate dehydrogenase, formaldehyde dehydrogenase and alcohol dehydrogenase by direct electron injection.

In this work we show the comparison of NADH assisted processes of enzymatic CO_2 reduction and experiments were NADH is substituted by a direct electron injection from an electrochemical process. The latter offers the opportunity to substitute NADH and therefore to decrease process costs significantly. To reduce reaction parameters we concentrate on the first step of the reduction of CO_2 to formate for the following work.

1.5 Enzymes as catalysts

In general enzymes are divided into six major groups according to the type of reaction they catalyze (see Figure 6).[26]

Class	Name	Type of reaction catalyzed		Example
1	Oxidoreductases	Transfer of electrons	$A^- + B \rightarrow A + B^-$	Alcohol dehydrogenase
2	Transferases	Transfer of functional groups	$A–B + C \rightarrow A + B–C$	Hexokinase
3	Hydrolases	Hydrolysis reactions	$A–B + H_2O \rightarrow A–H + B–OH$	Trypsin
4	Lyases	Cleavage of C–C, C–O, C–N and other bonds, often forming a double bond	$A–B \rightarrow A{=}B + X–Y$ (X, Y)	Pyruvate decarboxylase
5	Isomerases	Transfer of groups within a molecule	$A–B \rightarrow A–B$ (X Y, Y X)	Maleate isomerase
6	Ligases (or synthases)	Bond formation coupled to ATP hydrolysis	$A + B \rightarrow A–B$	Pyruvate carboxylase

Figure 6: Classification of enzymes into 6 major groups.[26]

Enzymes are biocatalysts that consist of a proteinic backbone with N- and C- terminus and an active site, which can be a metal ion form for example Zn or Mg.[27]

Some enzymes require no chemical groups for their activity other than their amino acid residues. Dehydrogenase, such as we are using in this work, as well as some other groups of enzymes require sacrificial co-enzymes as energy donors. Possible co-factors are either inorganic ions or complex organic molecules. Those cofactors can be a prosthetic group which is tightly bound to the enzyme or a coenzyme which is released from the enzyme's active site during the reaction. Coenzymes transport chemical groups or rather charges, substrates and formed products. However those co-enzymes are sacrificial and have to be regenerated.[27] Such regenerations require high energy inputs and are therefore difficult to achieve. In natural system regenerations are performed by additional catalyzed cycles.

Generally, dehydrogenases use the coenzyme NADH, Nicotinamide adenine dinucleotide, as electron acceptor.[24]

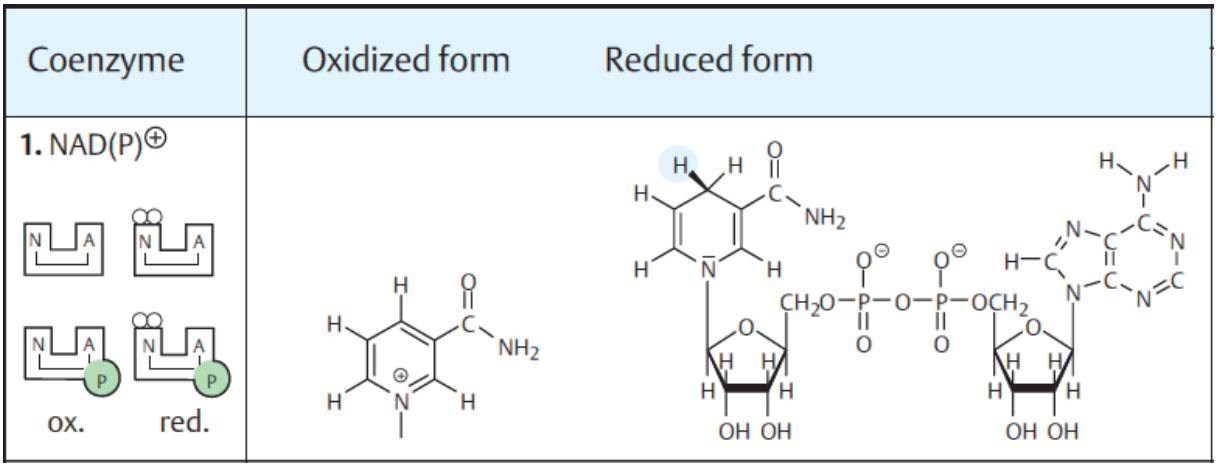


Figure 7: Structure of NADH (reduced form) and NAD^+ oxidized form.[28]

The active part of NADH is the nicotinamide heterocyclic ring. At the beginning of the reaction NADH is bound non-covalently by the enzymes and is released as its oxidized form NAD^+ after the reaction is finished. To be reusable for a reduction process NAD^+ has to be regenerated to NADH.

For an overall enzyme catalyzed process a simplified schematic can be written as

$E+S \rightleftharpoons [ES] \rightarrow E+P$

where E is the enzyme, S is the substrate and P is the product.
These stepwise reactions were proposed in 1913 by Leonor Michaelis and Maud Menten in their general theory of enzyme action.[24]
The first step of an enzymatic reaction is that the enzyme binds reversibly with the substrate to form an enzyme-substrate complex, which was first proposed by Victor Henri 1903. This step is relatively fast. In a slower second step the [ES] complex breaks down and the product is formed. The enzyme is unchanged and able to bind the next molecule of substrate.
According to this model, the second step limits the rate of the overall reaction. The overall rate is proportional to the concentration of the ES complex.
Michaelis and Menten derived an equation which describes the relationship between substrate concentration and reaction rate for a one-substrate enzyme catalyzed reaction[29].

Michaelis-Menten equation:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

V_0...initial velocity
V_{max}...maximum velocity
K_m...Michaelis constant, $(k_{-1}+k_2)/k_1$
[S]...initial substrate concentration

Enzymes show a very high specificity for their substrate. Two models have been investigated to explain the binding of a substrate to an enzyme. In the lock-and-key model the substrate and the active site of the enzyme are thought to fit together like a key into its lock. The two shapes are considered as rigid and fixed, and perfectly complement to each other. In the induced-fit model the binding of the substrate induces a conformational change in the active site of the enzyme.[26]

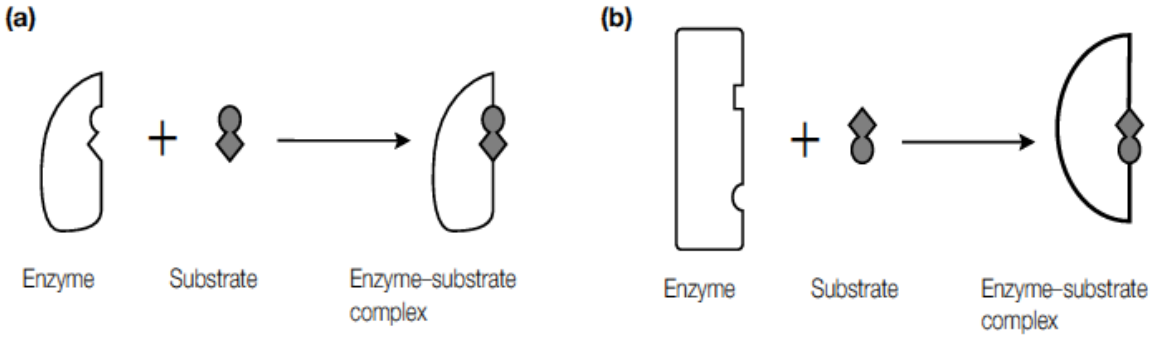


Figure 8: Binding methods of a substrate to an enzyme (a) lock-and-key model; (b) induced-fit model.[26]

For the utilization of enzymes it is essential to know that the three-dimensional conformation of the enzyme has an important effect on the catalytic activity. The catalytic activity of the enzyme is lost if the enzymes is denatured or broken down into its component amino acids[27]. Not only the conformation but also the active center plays an important role in enzyme catalysis. The active site of an enzyme is the part where the enzyme interacts with the substrate to form a substrate-enzyme complex. The active center consists of two sites which are lined with amino acid residues whose substituent groups binds the substrate and catalyze its chemical formation.[30] One site participates in the catalytic reaction whereas the other site controls the substrate specificity of the enzyme.

Enzymes offer several advantages, but depict also some unfavourable characteristics. On the one hand enzymes show the ability to catalyze a reaction under very mild conditions in neutral aqueous solution at normal temperatures, pressures[30] and pH values[27]. Moreover, they possess a very high specificity for their substrate, which makes them especially suitable for CO_2 recycling. On the other hand, enzymes are unstable molecules, due to their proteinic structure and they cannot be used in organic solvents or at elevated temperatures. Further, synthesis of enzymes is complex and expensive. Therefore it is necessary to optimize experiments towards reusability and stability of the biocatalysts.

1.6 Immobilisation of enzymes

As mentioned in section 1.4 applications of enzymes are limited due to high costs of enzyme synthesis. To benefit from the advantages of enzymes such as high yield, selectivity and biocompatibility those catalysts need to be separated from the product after reduction reactions. In this work the idea was to combine favourable properties of enzymes and heterogeneous catalysis. Immobilisation of enzymes in certain matrices provides reusability and stability of enzymes.

Considering these facts for enzyme immobilisation it is necessary to retain the native structure as far as possible. Consequently, immobilisation should be performed under very mild and well-controlled conditions, as the catalytic activity is changed if the amino acid residues at the active centre or the tertiary structure are altered.[30]

For enzymes following immobilisation techniques are known:

1. Carrier binding method: the binding of enzymes to water-insoluble carriers
2. Cross-linking method: intermolecular cross-linking of enzymes by means of bifunctional or multifunctional reagents
3. Entrapping method: incorporation of enzymes into the lattice of a semipermeable gel or enclosing the enzymes in a semipermeable polymer membrane where the enzyme itself does not bind to the gel matrix or membrane.

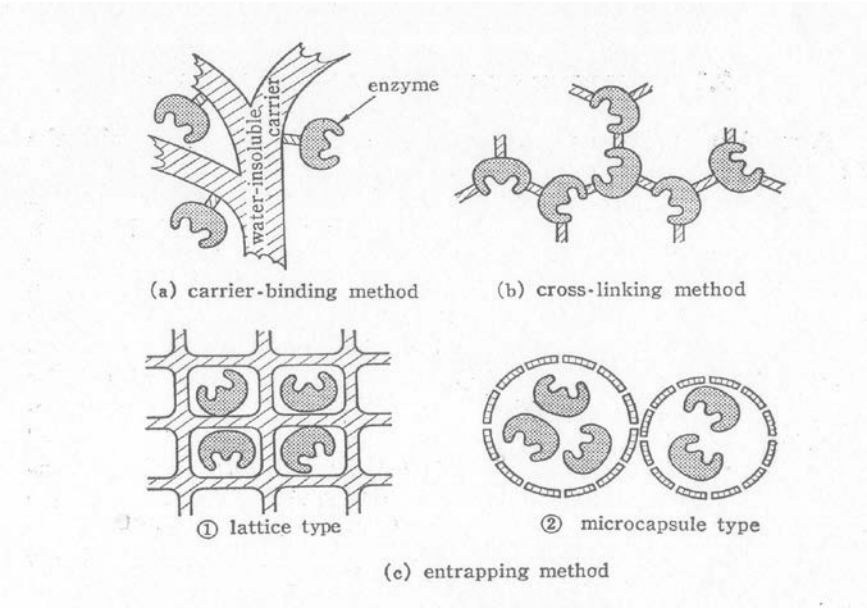


Figure 9: Different methods for enzyme immobilisation a) carrier binding method, b) cross-linking method, c) entrapping method.[30]

As immobilisation matrices several materials have been investigated. Aresta et al. investigated agar as well as polyacrylamide, pumice and zeolithe materials concerning stability and activity of the carboxylase enzyme to be used for the synthesis of benzoic acid from phenol and carbon dioxide.[21] Further different kinds of sol-gels were investigated for the immobilisation of enzymes. Obert and Dave presented the immobilization of dehydrogenase enzymes for chemical CO_2 reduction in silica sol-gel matrices.[22] The most frequently used technique for the immobilisation of enzymes is the encapsulation in alginate containing gels like Heichal-Segal presented in 1995.[31] Based on this idea the groups of Lu and Wu were using a hybrid alginate silicate gel for the immobilisation of dehydrogenase enzymes.[32] The cross-linking of silica with the alginate matrix leads to a compact and porous composite with good diffusion characteristics. This immobilisation matrix ensures the stability of the enzyme without inhibiting the active site and makes it therefore reusable for several reaction processes.

2. Experimental Part

All chemicals were used as received. Formate dehydrogenase (from candida boidinii, lyphilized powder, 5-15 units/mg protein) was purchased from Sigma Aldrich. NADH, Tetraethylorthosilicate (TEOS), Tris(hydroxymethyl)-aminomethan (TRIS) and PEDOT:PSS were as well obtained from Sigma Aldrich. Concentrated Hydrochloric acid (37%) was purchased from Fischer Scientific. The pH of the TRIS buffer solution was adapted with 1M HCl to pH 7.64 according to the pH optimum of formate dehydrogenase between 7.5 and 8.5. Products from CO_2 reduction experiments were analysed by manual injection of liquid samples in capillary ion chromatography (CAP-IC, Dionex ICS 5000, AS 19 analytical column). Identification of the product in CAP-IC was done by using external standards with different concentration of formate in buffer solution.

2.1 Non-electrochemical experiments

2.1.1 Preparation of Alginate-Silicate Beads

5 mg of $F_{ate}DH$ were dissolved in 1 mL 0.05 M TRIS-HCl buffer and added to a mixture of 0.1 g alginic acid sodium salt in 4 mL 18mΩ water and 1.47 mL TEOS to obtain a 2% alginate solution.
For precipitation and to achieve high surface areas, the solution was slowly dropped into a 0.2 M $CaCl_2$ solution with a 5 mL syringe to form beads of 2-3 mm diameter. Immediate gelation could be observed. After 30 minutes of congelation the beads were filtered and rinsed with 18mΩ water.

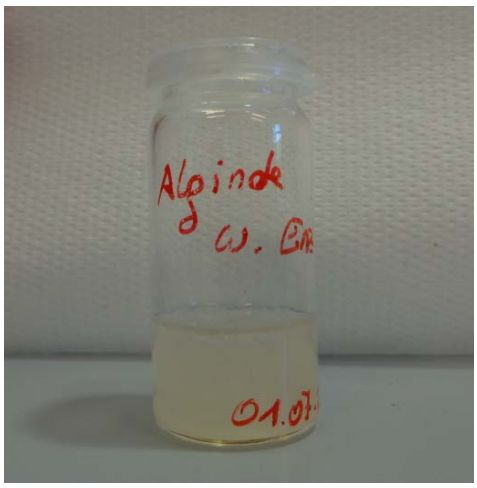


Figure 10: Liquid gel matrix for preparation of alginate-silicate beads.

Figure 11: Precipitated alginate-silicate beads.

2.1.2 CO_2 reduction experiment (Alginate-Silicate Beads)

For the CO_2 reduction experiment the alginate-silicate beads were put into a three-necked flask containing 3 mL TRIS-HCl buffer of pH 7.64. Approximately 5-10 mg NADH were filled into a small vial. The vial was connected to the flask via a tube and not added directly to prevent NADH oxidation. After saturating the reaction cell with N_2 NADH was added. For proof of the activity of the enzymes and successful reduction of CO_2 to formate the system was purged with N_2 and CO_2 respectively after NADH addition. Before the reaction was started and after about two hours of CO_2 purging samples of the solution were taken. Samples before and after the experiments were analysed and compared using CAP-IC.

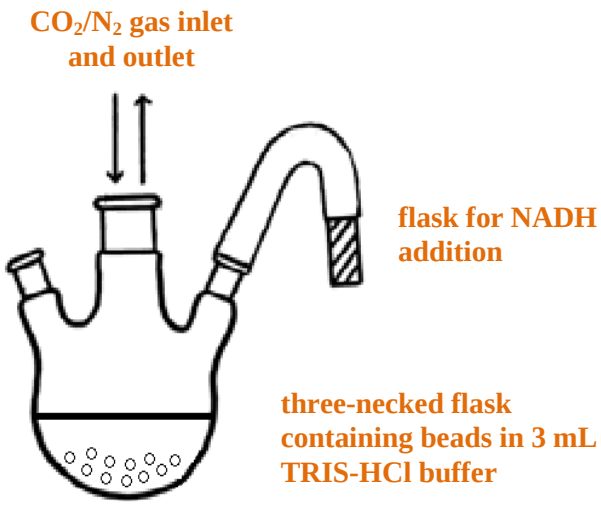


Figure 12: Simplified setup used for the non-electrochemical experiments to purge alginate-silicate beads with N_2/CO_2. Consisting of a three-necked flask containing the beads, a flask for addition of NADH, a N_2/CO_2 inlet as well as a gas outlet.

2.1.3 Preparation of Alginate Beads containing PEDOT:PSS

The preparation of alginate beads with PEDOT:PSS was performed in the same way as for the beads in section 2.1.1. For adding PEDOT:PSS to the mixture alginic acid sodium salt was dissolved in 4 mL filtered PEDOT:PSS instead of 18mΩ water. Further steps were performed identically (section 2.1.1).

2.1.4. CO_2 reduction experiment (Alginate-Silicate Beads containing PEDOT:PSS)

CO_2 reduction was performed as it was done for enzyme beads without PEDOT:PSS. Again samples of the N_2 purged system were compared to samples of the CO_2 purged system before and after the experiment by using CAP-IC analysis.

2.2 Electrochemical experiments

Electrochemical measurements were performed in a one compartment cell with JAISLE Potentiostat-Glavanostat IMP 88 PC using pristine as well as modified Pt and carbon felt as working electrode. Further Pt foil operated as counter electrode and Ag/AgCl/KCl as reference electrode. A buffer solution of 0.05 M TRIS-HCl with pH=7.64 was used as electrolyte. Cyclic voltammograms (CV) were recorded after 10 min of N_2 and CO_2 purging respectively for electrochemical characterisation. The potential was sweeped between 300 mV and –1900 mV vs. Ag/AgCl at a scan rate of 50 mV/s. Electrolysis experiments were conducted in potentiostatic mode at -1900 mV vs Ag/AgCl for 1.5 h after 30 min N_2 and CO_2 saturation respectively for comparison. Samples from electrolysis experiments of the CO_2 saturated system are compared to samples of the N_2 saturated system to proof product generation from CO_2 reduction.

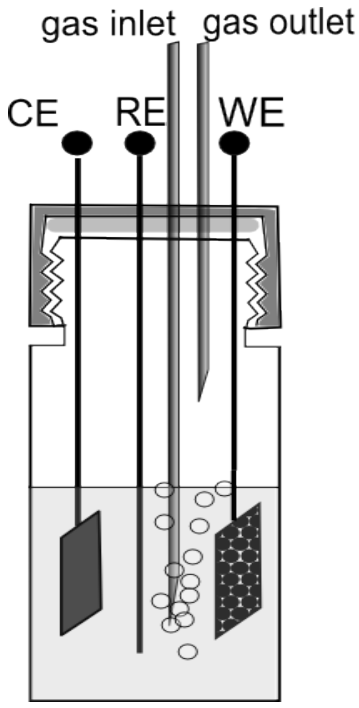


Figure 13: One compartment cell consisting of a counter electrode, reference electrode, gas inlet, gas outlet and a working electrode

2.2.1 Preparation of coated electrodes

For the immobilisation of enzymes on an electrode also alginate-silicate hybrid gels were used. For a better adhesion on the electrode the viscosity of the alginate solution was therefore increased to 4%. Experiments were conducted using pristine alginate-silicate sol-gels as well as PEDOT:PSS modified gels.

The platinum or carbon felt, respectively, was alternately dipped into the alginate solution and the $CaCl_2$. After dipping into the $CaCl_2$ solution the electrode was rinsed with 18mΩ water. These steps were as long repeated until the electrode was fully coated. For complete gelation of the alginate solution the electrode was immersed in the $CaCl_2$ solution for 30 minutes.

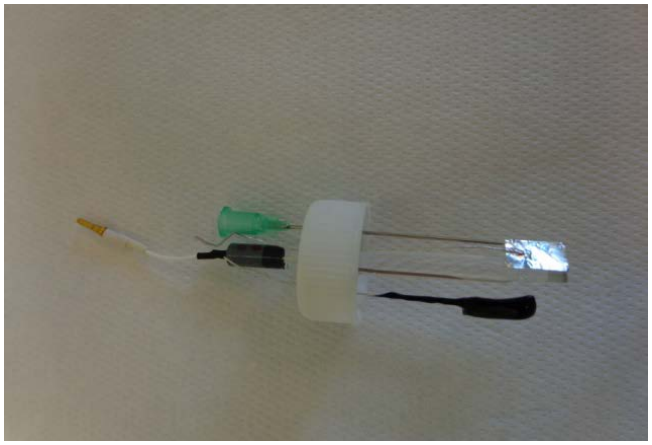


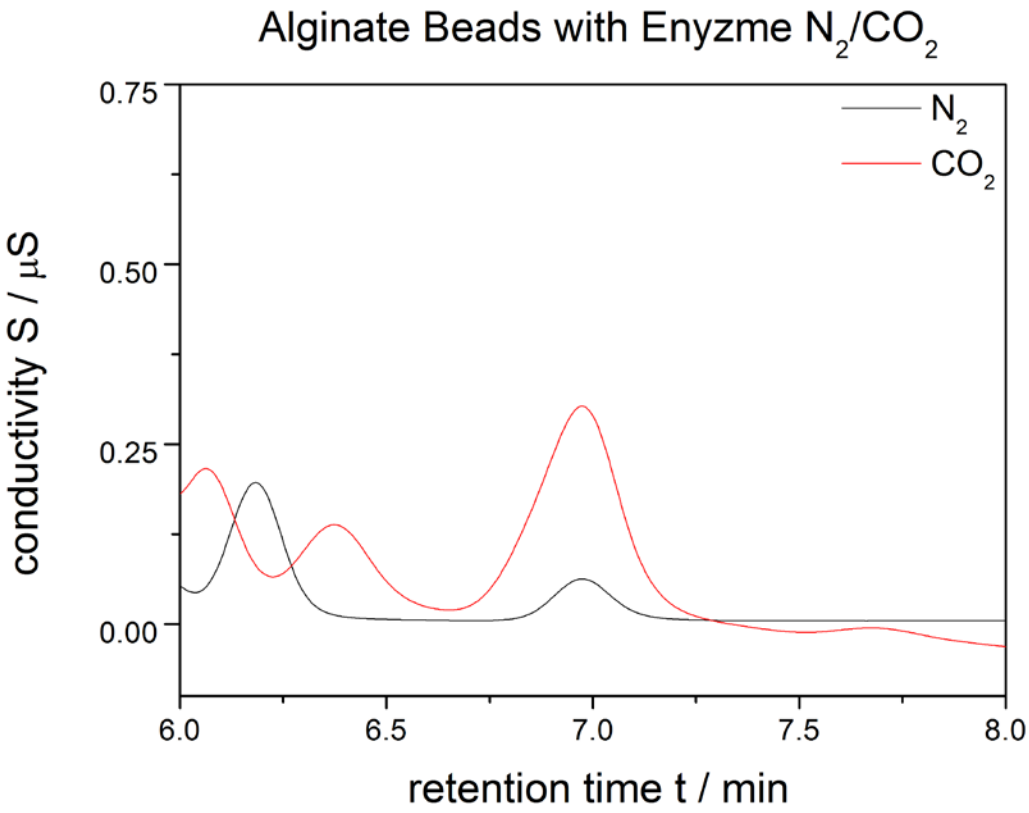
Figure 14: Platinum working electrode coated with PEDOT:PSS-alginate matrix (black); platinum foil counter electrode and reference electrode Ag/AgCl/KCl

3. Results and Discussion

3.1 Non-electrochemical experiments

3.1.1 Alginate-Silicate Beads

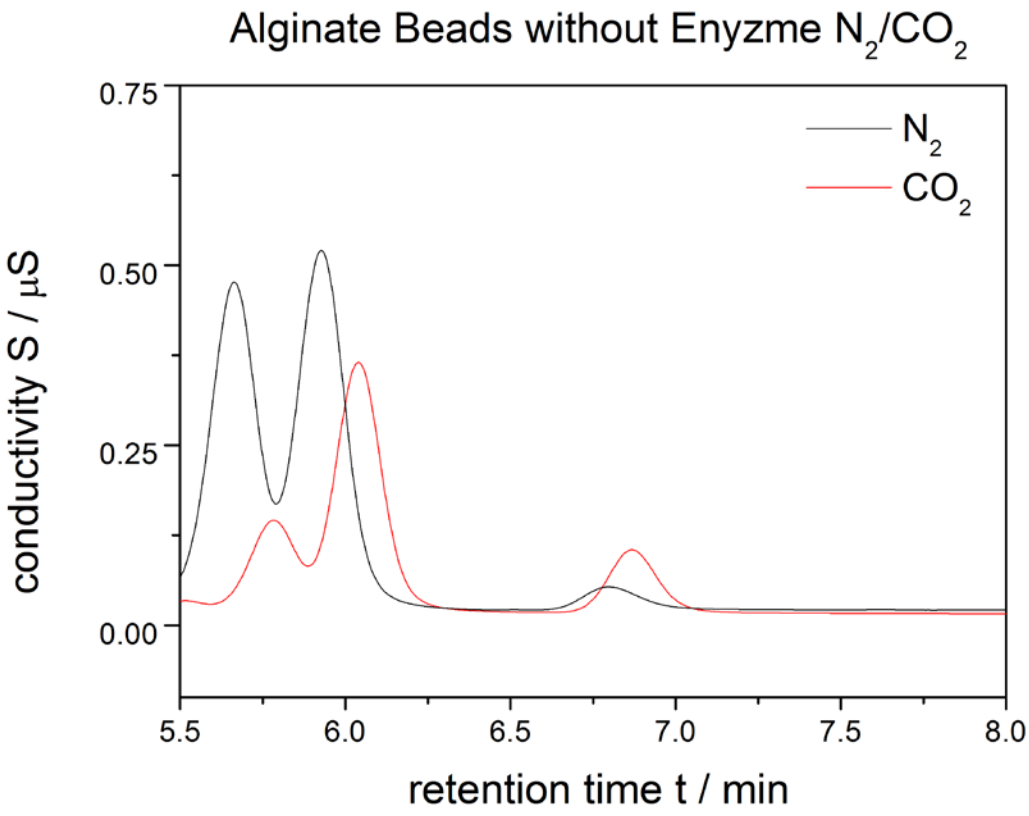
Graph 1 shows the comparison of samples after saturating the cell with N_2 and CO_2 respectively. At the characteristic retention time of formate at t=6.9 min a growing peak is observed after purging the system with CO_2. The small peak observed for the sample after N_2 purging is also observed for water injections and is therefore attributed to device contamination. The practical yield of formate is 7.38 ppm (0.16 mmol/L). To optimize the yield of formate production duration of purging with CO_2 can be increased as well as the amount of NADH added, which are the limiting factors for formate generation (see Figure 4).



Graph 1: Comparison of IC measurements of alginate-silica beads containing $F_{ate}DH$. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup. At retention time t=6.9 min formate production is obvious for the CO_2 purged system.

In Graph 2 the comparison of samples without enzymes is shown. Compared to Graph 1 formate peaks at retention time t=6.9 min are much smaller. Further for the peak of the N_2 purged system similar peak areas are determined as for the peak of the CO_2 purged system and therefore correlate with impurity fluctuations of column and laboratory equipment. At retention times t=5.5 min to t=6.3 min peaks indicate common contaminations of acetate and fluoride from laboratory equipment such as syringes, filters and non-purified water. To avoid these impurities for further experiments syringes and filters used were first rinsed with 18mΩ water several times and then rinsed with the sample solution. For diluting samples and for buffer solution as well ultrapure 18 mΩ water was used only.

Summarized, as expected for experiments without enzyme no formate peak increase can be observed.

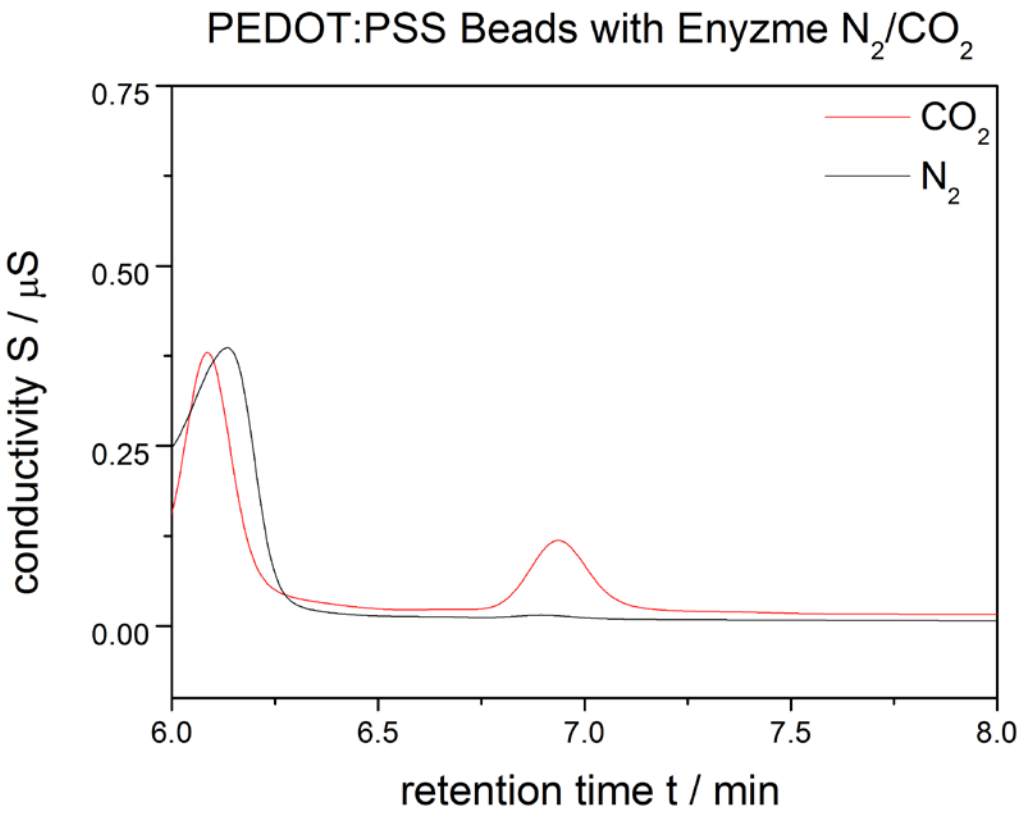


Graph 2: Comparison of IC measurements of alginate-silica beads without $F_{ate}DH$. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup.

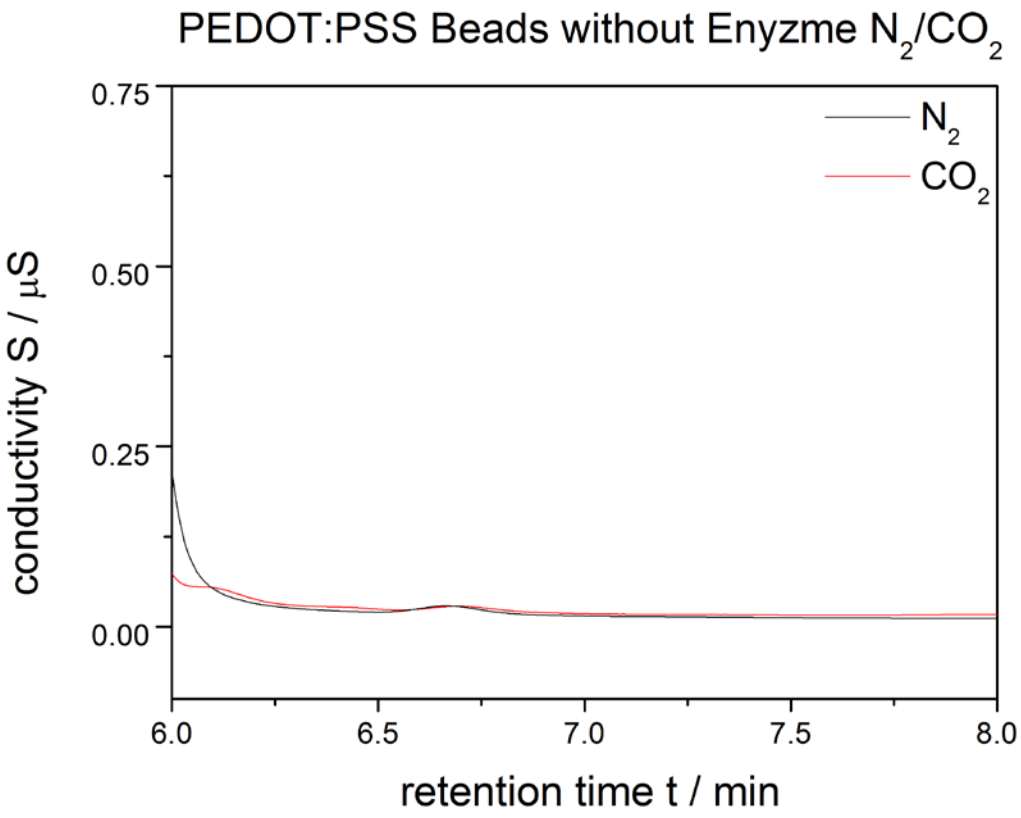
3.1.2 Alginate-PEDOT:PSS Beads

Also for the PEDOT:PSS modified alginate beads a peak at t=6.9 min in the CO_2 purged system is clearly visible as shown in Graph 3. It can be concluded that PEDOT:PSS does not deactivate or even inhibit the active centre of the enzyme. The experiment yielded approximately 2.8 ppm (0.06 mmol/L) of formate which is in the same order of magnitude than determined for beads without PEDOT:PSS.

As expected the system without enzyme did not produce any formate (see Graph 4).



Graph 3: Comparison of IC measurements of PEDOT:PSS beads containing $F_{ate}DH$. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N2 purging of the setup. At retention time t=6.9 min formate production is obvious for the CO_2 purged system.

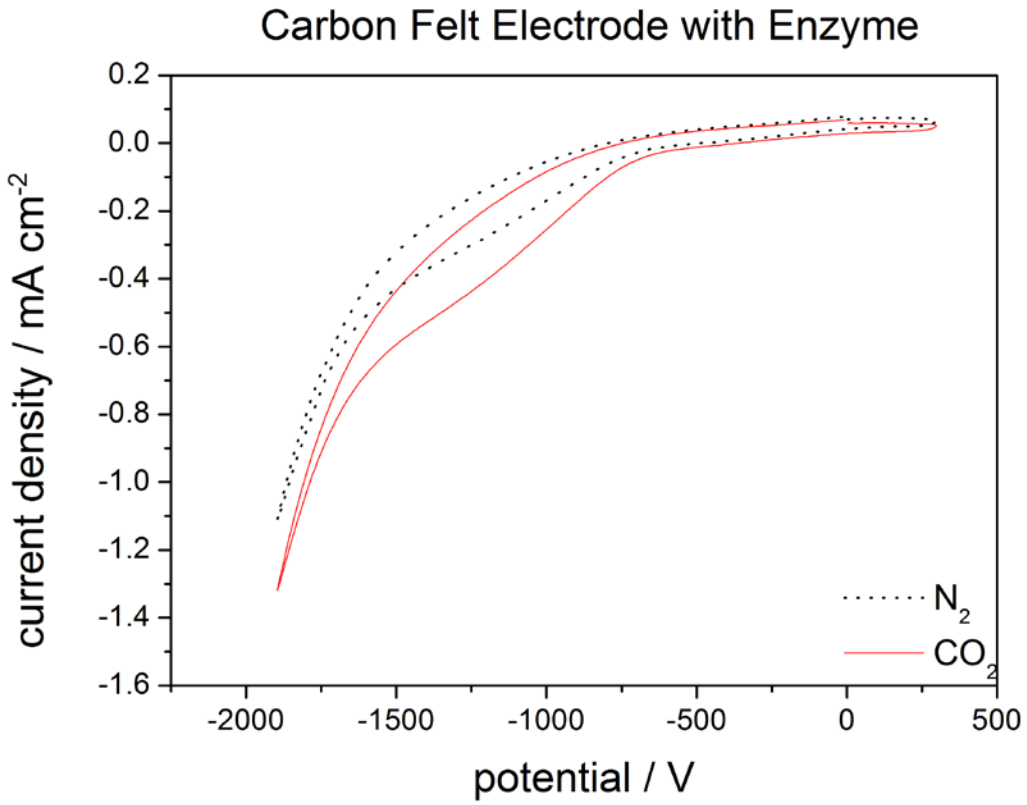


Graph 4: Comparison of IC measurements of PEDOT:PSS beads without F_{ate}DH. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup.

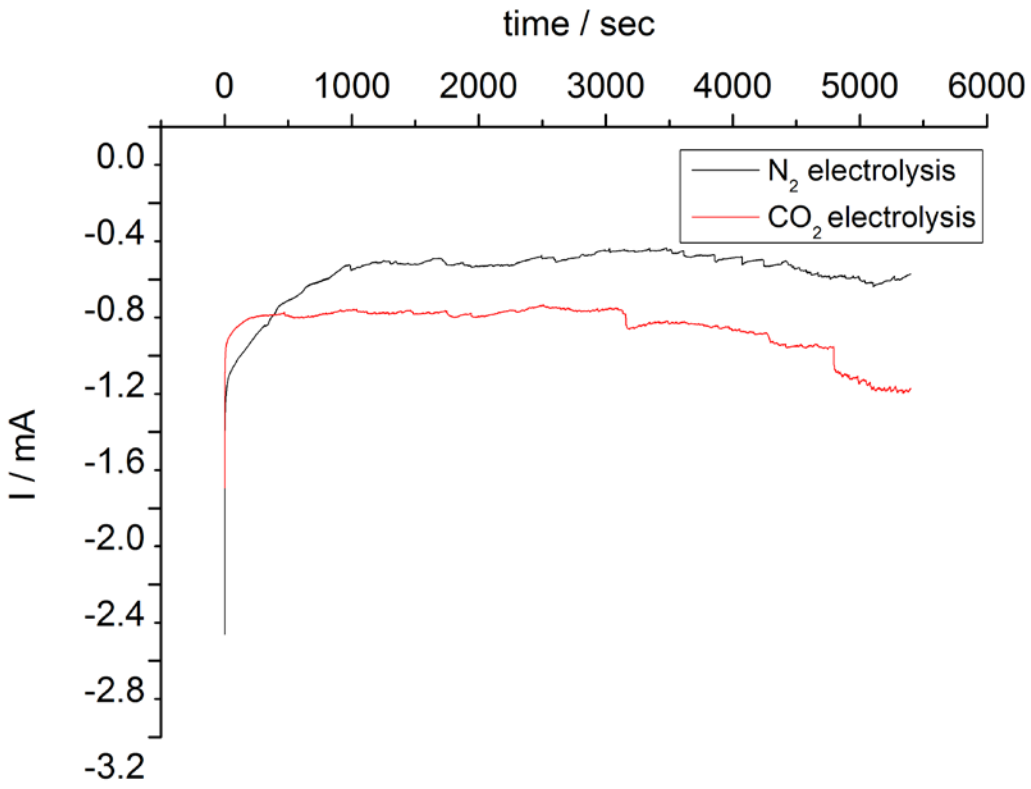
3.2 Electrochemical Experiments

3.2.1. Alginate Carbon Felt Electrode

In Graph 5 the CVs of a carbon felt electrode modified with enzymes are shown. Compared to the CV after N_2 purging an increase in current density from -1500 mV vs. Ag/AgCl is observed for the system after CO_2 purging. This indicates reduction of CO_2 at an overpotential of 1.1V. Therefore electrolysis experiments were conducted in potentiostatic mode at -1.8 V vs. Ag/AgCl.

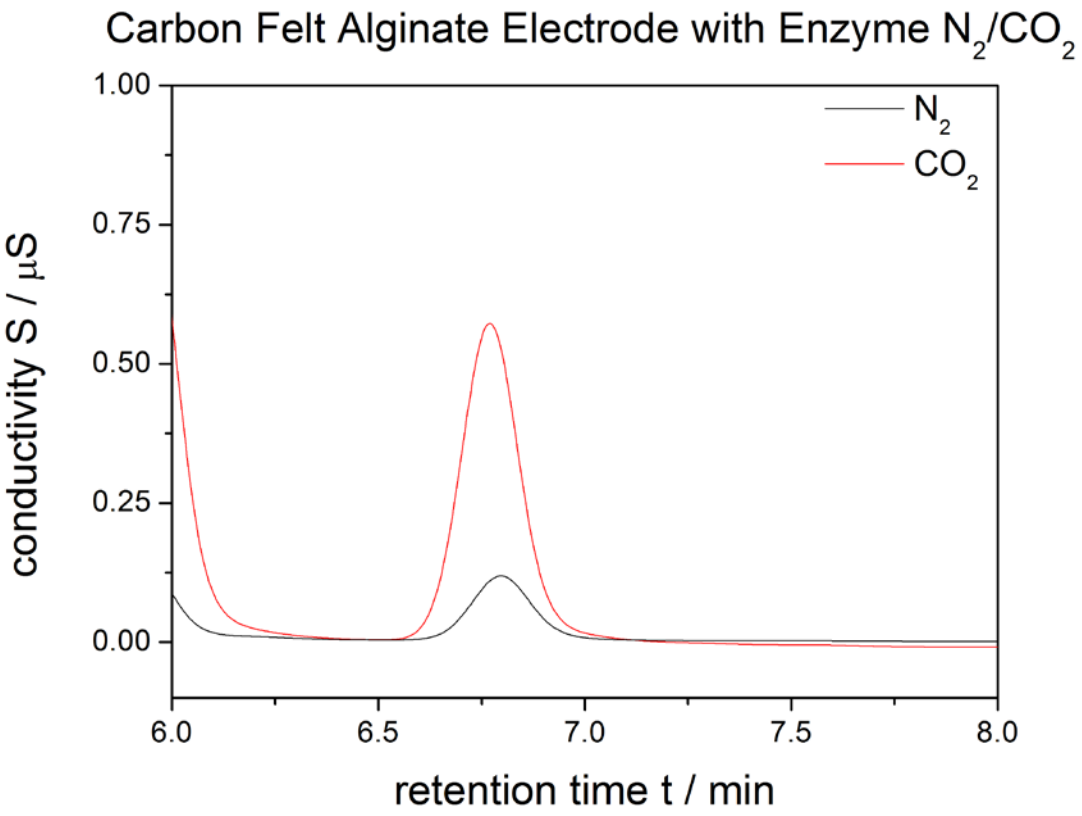


Graph 5: Comparison of cyclic voltammograms of CF electrode with immobilized alginate containing F_{ate}DH. The black curve depicts CV after 10 min of N_2 purging, the red curve shows the CV after CO_2 purging with increase in reductive current.



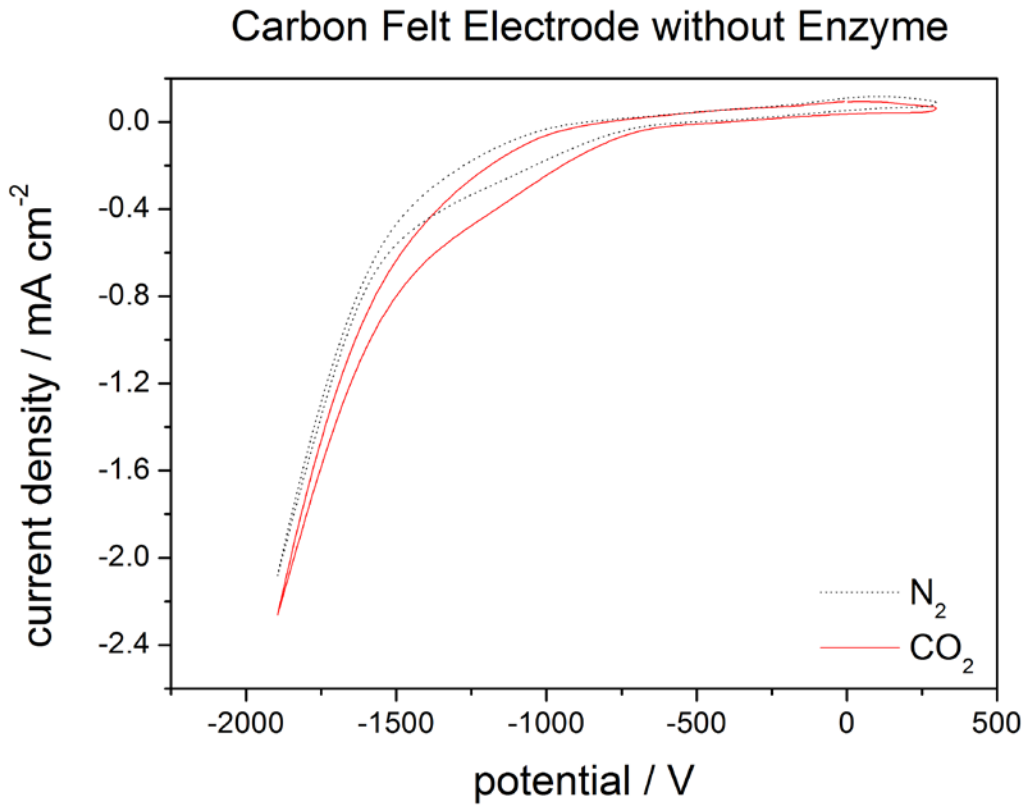
Graph 6: Comparison of the measured current on the time of electrolysis. The red curve depicts the current of electrolysis after CO_2 purging, and the black curve shows the current of electrolysis after N_2 purging.

The chromatograms of samples after electrolysis experiments in Graph 7 shows a distinct increased peak at the retention time of formate at t=6.8 min for the CO_2 purged system compared to the N_2 purged system. This result shows for the first time that $F_{ate}DH$ can be addressed directly without requiring any sacrificial co-enzyme. Electrons are provided by the electrode in the electrochemical system. Hydrogen is provided by the aqueous buffer solution. This direct electrochemical reduction of CO_2 by an immobilized enzyme gives the opportunity for a highly selective CO_2 conversion at decreased costs. For this process around 5.5 ppm (0.12 mmol/L) at 3.71 Coulombs were detected which corresponds to a Faradaic efficiency of around 10%. The peak at t=6.0 min again correlates to acetate contamination. Moreover, Graph 6 confirms the production of formate.

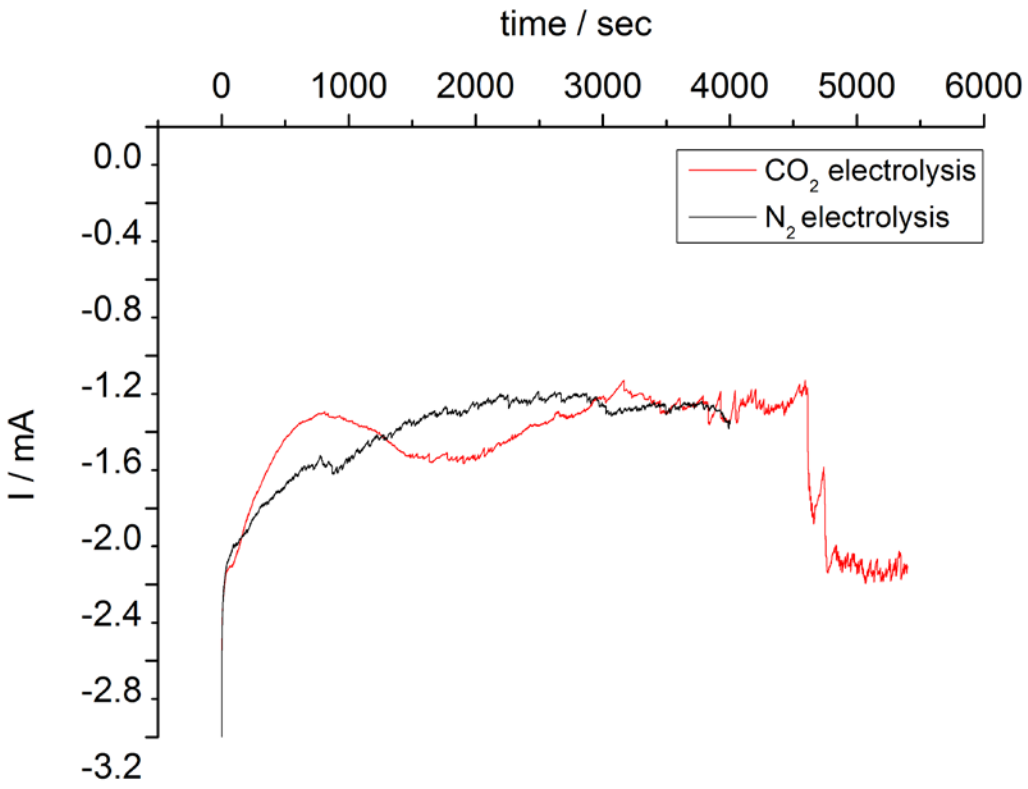


Graph 7: Comparison of IC measurements of carbon felt alginate electrode containing F_{ate}DH. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup. At retention time t=6.8 min formate production is obvious for the CO_2 purged system.

In Graph 8 also for the pristine alginate electrode without enzymes a small reductive current after CO_2 purging was observed. Due to this result an electrolysis experiment as well as CAP-IC analysis were performed which did not feature any CO_2 reduction products. As expected formate was not detected since there was no active enzyme used in the experiment.

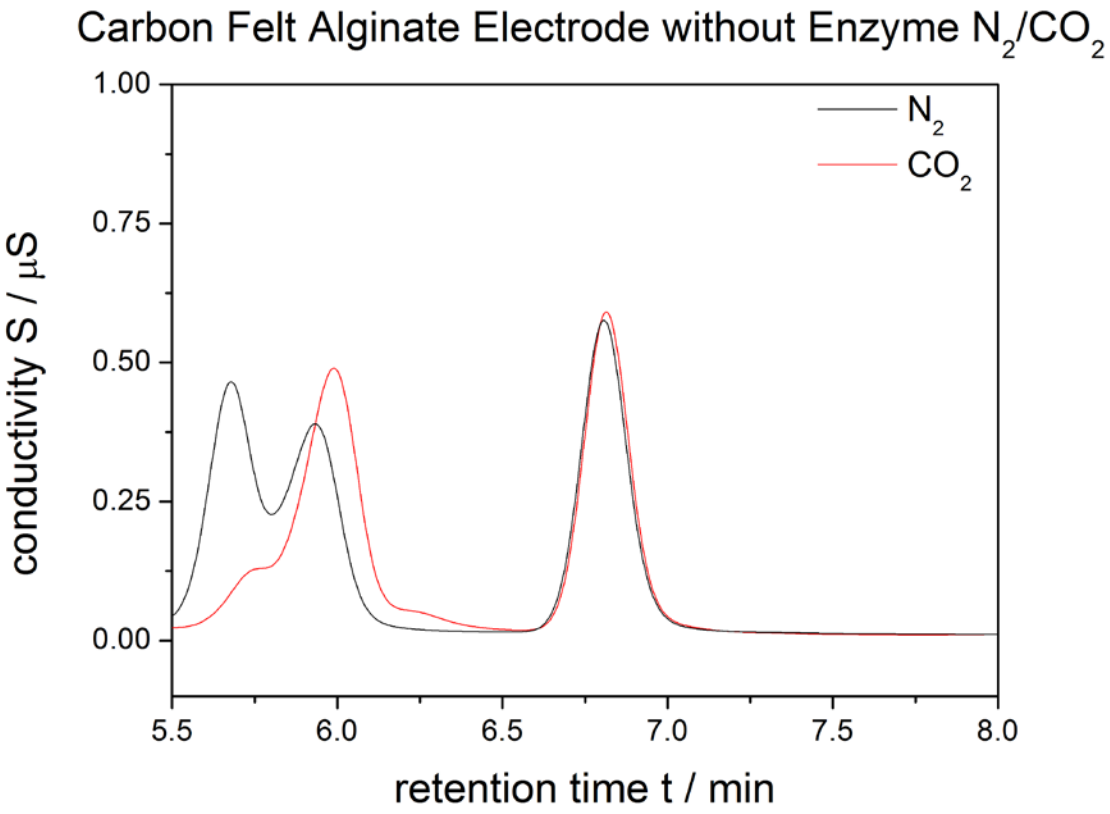


Graph 8: Comparison of cyclic voltammograms of CF electrode with immobilized alginate containing $F_{ate}DH$. The red curve depicts CV after 10 min of N_2 purging, the red curve shows the CV after CO_2 purging with increase in reductive current



Graph 9: Comparison of the measured current on the time of electrolysis. The red curve depicts the current of electrolysis after CO_2 purging, and the black curve shows the current of electrolysis after N_2 purging.

As expected, Graph 10 shows no visible change of the formate peak. For this experiment no enzymes were immobilized. The peak height of the formate peak is assumed to be increased due to fluctuation problems of the conductivity detector used for the CAP-IC device.



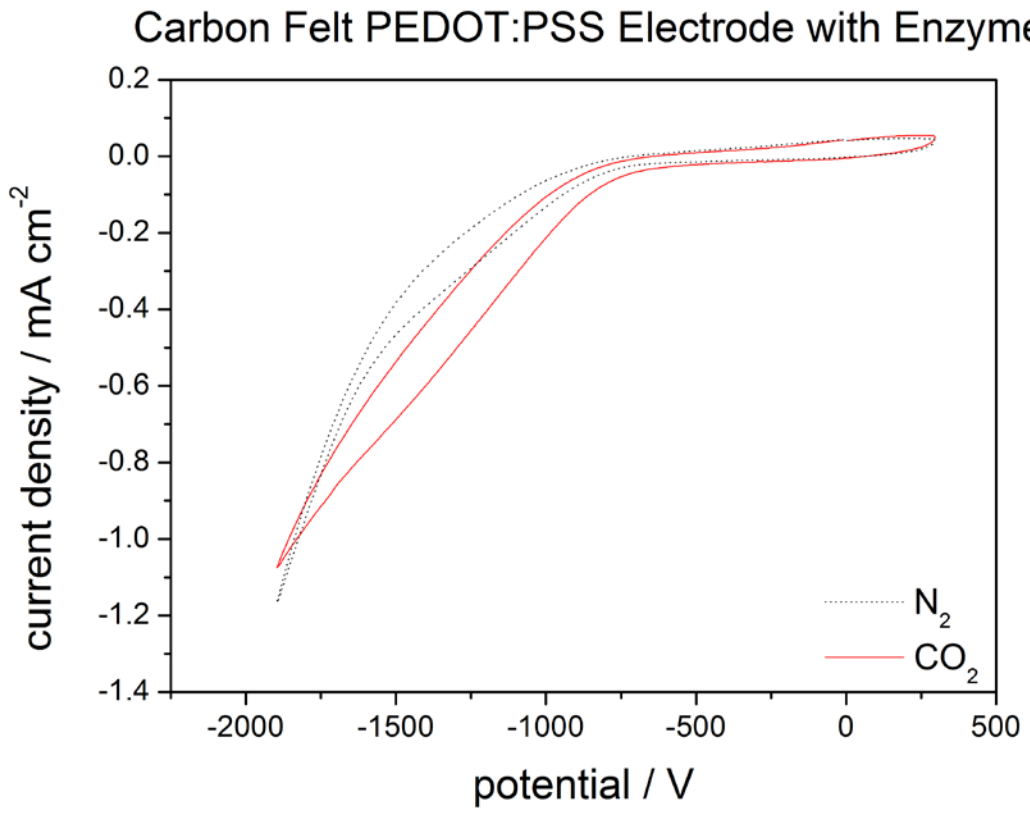
Graph 10: Comparison of IC measurements carbon felt alginate electrode without F_{ate}DH. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup.

3.2.2. PEDOT:PSS Alginate Carbon Felt Electrode

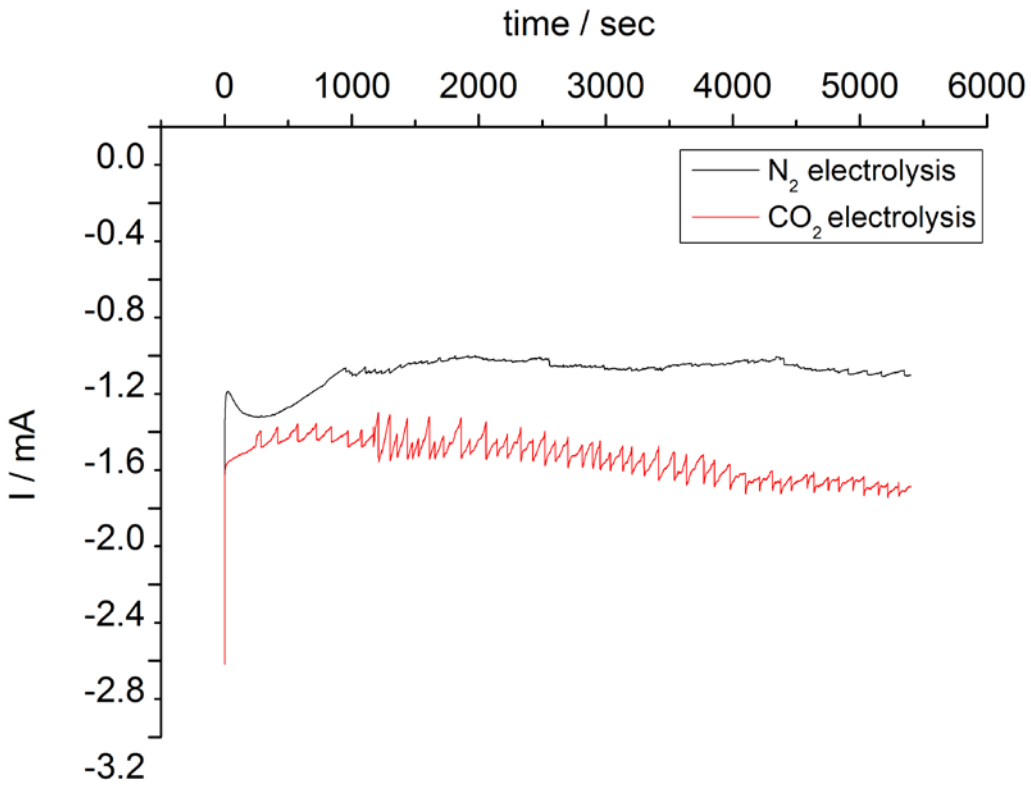
For the chromatograms of the carbon felt PEDOT:PSS electrodes with and without enzyme the formate peak reaches nearly the same height (see Graph 13 and Graph 16). However, there is also a peak at the retention time of formate observed for the sample before the reaction was started. This might be due to column contamination from former experiments.

We assume formate production of approximately 9.8 ppm (0.21 mmol/L) which correlated to a faradaic efficiency of approximately 10%.

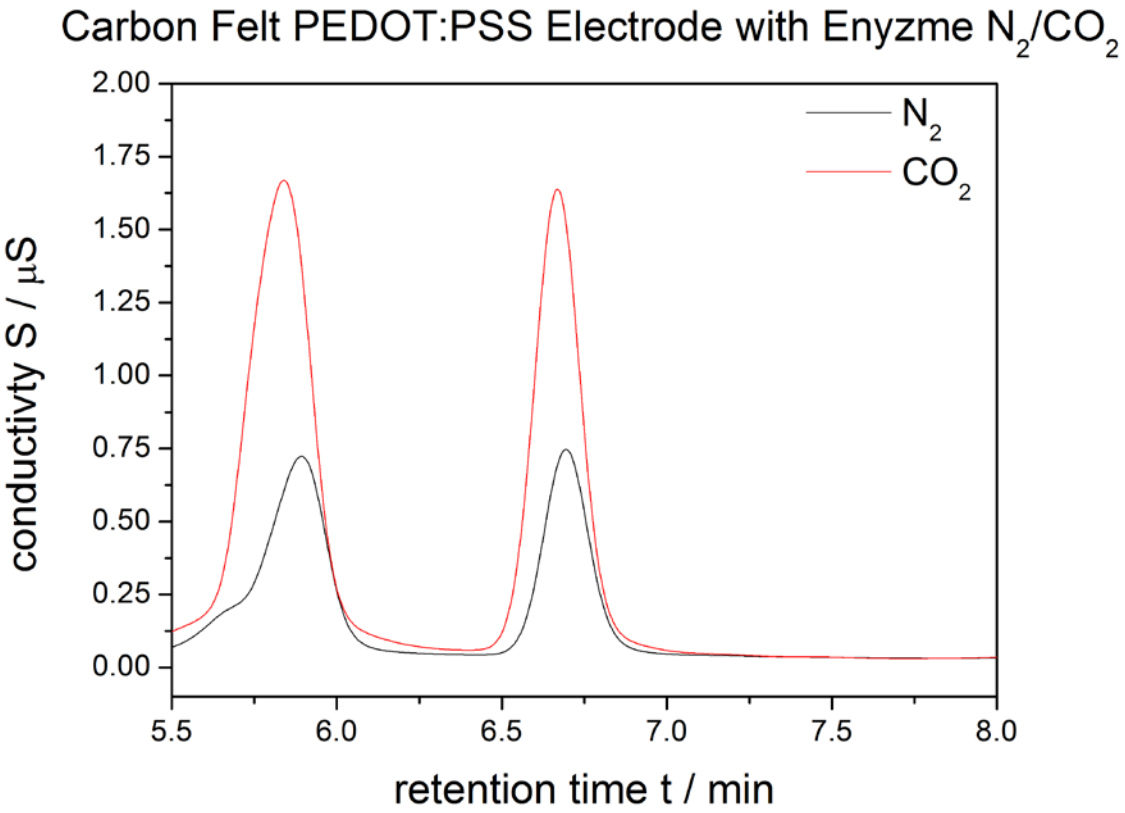
Furthermore, Graph 12 and Graph 15 depict formate production for the experiments with and without enzyme.



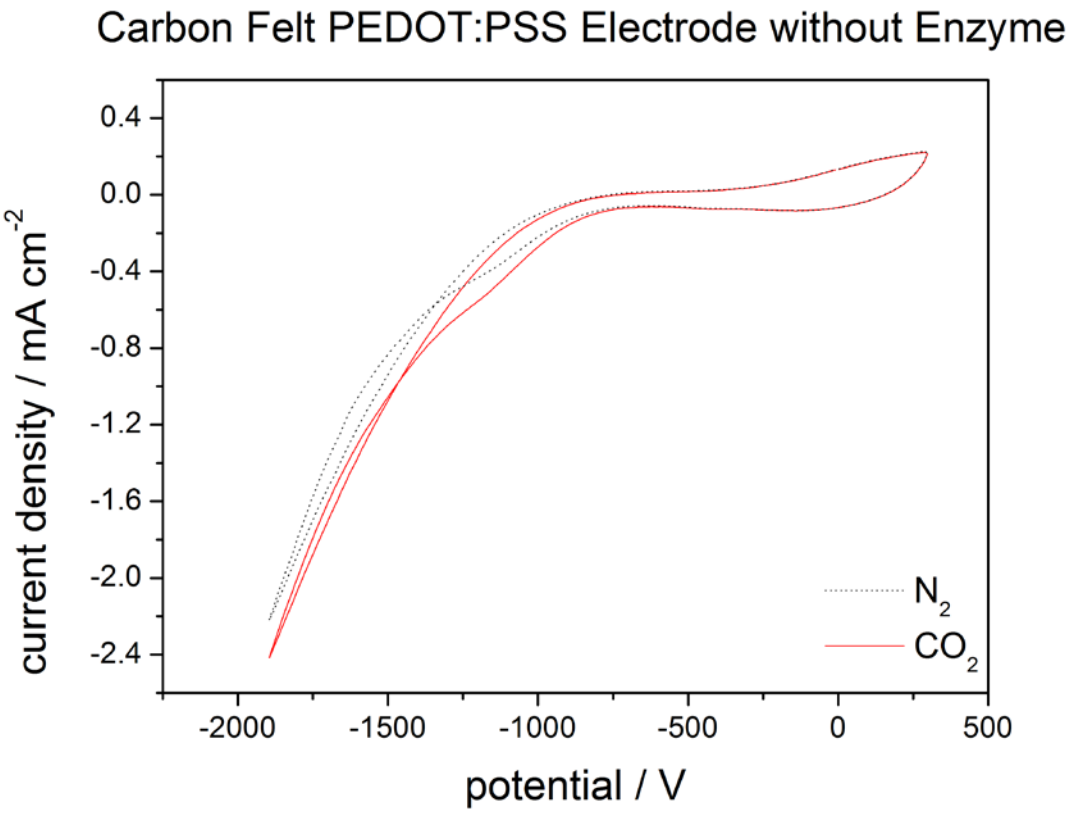
Graph 11: Comparison of cyclic voltammograms of CF PEDOT:PSS electrode with immobilized alginate containing $F_{ate}DH$. The red curve depicts CV after 10 min of N_2 purging, the red curve shows the CV after CO_2 purging.



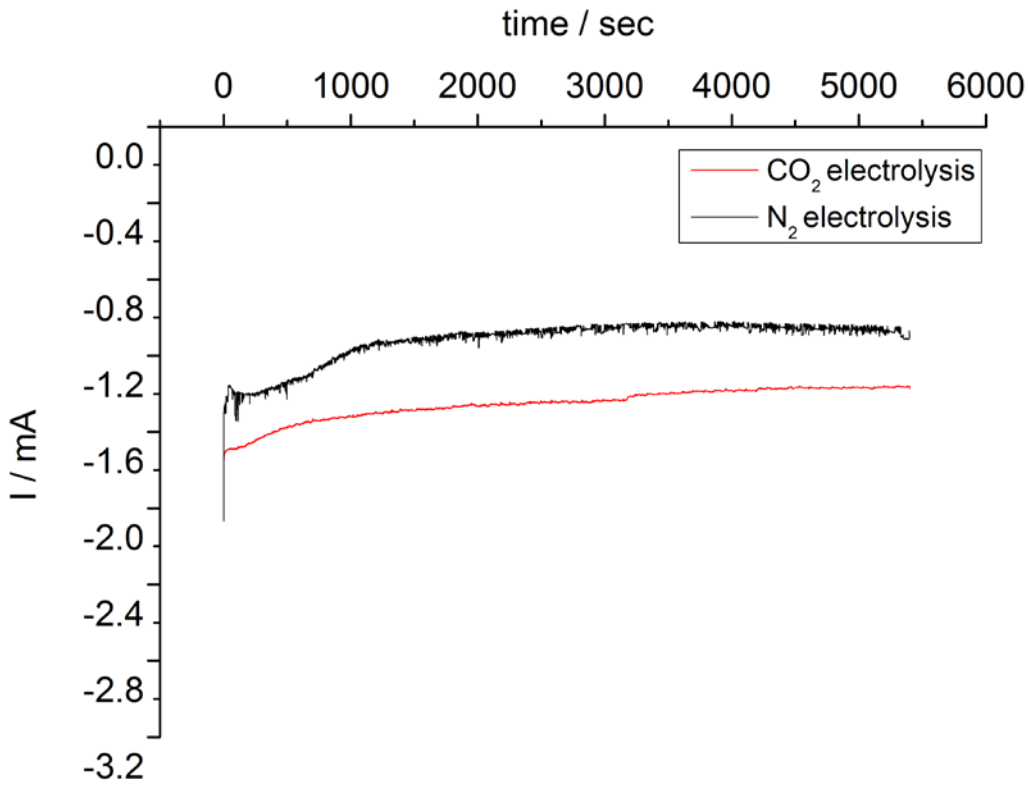
Graph 12: Comparison of the measured current on the time of electrolysis. The red curve depicts the current of electrolysis after CO_2 purging, and the black curve shows the current of electrolysis after N_2 purging.



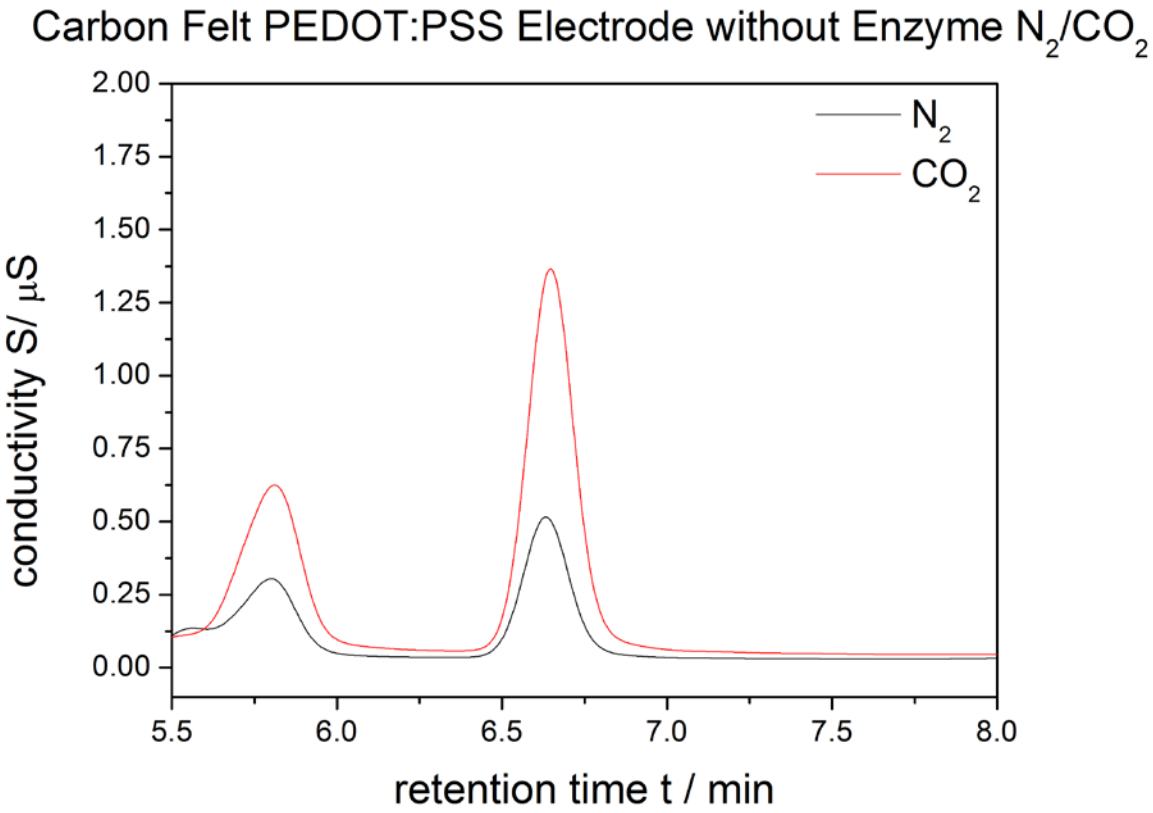
Graph 13: Comparison of IC measurements of carbon felt PEDOT:PSS electrode containing F_{ate}DH. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup. At retention time t=6.7 min formate production is obvious for the CO_2 purged system.



Graph 14: Comparison of cyclic voltammograms of CF PEDOT:PSS electrode with immobilized alginate containing F_{ate}DH. The red curve depicts CV after 10 min of N_2 purging, the red curve shows the CV after CO_2 purging.



Graph 15: Comparison of the measured current on the time of electrolysis. The red curve depicts the current of electrolysis after CO_2 purging, and the black curve shows the current of electrolysis after N_2 purging.



Graph 16: Comparison of IC measurements of carbon felt PEDOT:PSS electrode without F_{ate}DH. The red curve depicts the IC analysis after CO_2 purging, the black curve indicates the IC analysis after N_2 purging of the setup.

4. Conclusion

This work presents two different ways to convert CO_2 to formate by using formate dehydrogenase as biocatalyst. Non-electrochemical as well as electrochemical experiments delivered formate generation from CO_2 reduction experiments using immobilized $F_{ate}DH$. These results support the approach of immobilizing dehydrogenase enzymes in hybrid alginate-silicate sol-gel matrices as convenient method to immobilize enzymes and stabilize them at the same time. The yields of formate generation of the non-electrochemical experiments using pristine alginate-silicate sol-gels and PEDOT:PSS modified gels are in the same order of magnitude. To optimize the yield of formate production duration of purging with CO_2 can be increased as well as the amount of NADH added, which are the limiting factors for formate generation. Moreover, it can be concluded that PEDOT:PSS does not deactivate or inhibit the active centre of the enzyme. However, PEDOT:PSS modified gels still need to be improved by conducting further experiments with varying amounts of PEDOT:PSS.

As a next step the CO_2 reduction process using dehydrogenase enzymes has to be extended by using the enzyme cascade with $F_{ate}DH$, $F_{ald}DH$ and ADH to obtain methanol.

The preliminary results of this work for the two electron reduction (one step) of CO_2 to formate using $F_{ate}DH$, show for the first that enzymes can be addressed directly without any sacrificial mediator or co-enzyme needed. This offers the opportunity of NADH substitution and provides therefore a highly selective way of CO_2 reduction and sustainable energy conversion. Experiments will be carried out further to investigate the influence of PEDOT:PSS on enzymatic CO_2 reduction using electrochemistry.

References

[1] Boxun Hu, Curtis Guild, Steven L. Suib, Journal of CO_2 Utilization **2013**, 18-27

[2] Kai Man Kerry Yu, Igor Curcic, Joseph Gabriel, and Shik Chi Edman Tsang, ChemSusChem **2008**, 1, 893-899

[3] J.C.M. Pires, F.G. Martins, M.C.M. Alvim-Ferraz, M.Simoes, Chemical Engineering Research and Design **2011**, 89, 1446-1460

[4] Brett P. Spigarelli, S. Komar Kawatra, Journal of CO_2 Utilization **2013**, 1, 69-87

[5] R.F. Keeling, Science **2008**, 319

[6]B. Chameides, Environmental Defense Fund, **2007**, http://blogs.edf.org/climate411/2007/06/29/human_cause-3/

[7] F. Johnsson et al, THERMAL SCIENCE **2012**, 16, 655-668

[8] Paul Johnston, David Santillo, Greenpeace Research Laboratories, University of Exeter, Prince of Wales Road, Exeter, EX4 4PS

[9] M. Aresta, Carbon Dioxide as Chemical Feedstock, **2010**, WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim

[10] M. Aresta, Carbon Dioxide Recovery and Utilisation, **2010**, Kluwer Academic Publishers

[11] G.A. Olah, A. Goeppert, G.K.S. Prakash, Beyond Oil and Gas: The Methanol Economy, **2009**, WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim

[12] B. Kumar, M. Llorente, J. Froehlich, T. Dang, A. Sathrum, C.P. Kubiak, Annu. Rev. Phys. Chem., **2012**, 63, 541-569

[13] J.Hawecker, J.-M. Lehn, R. Ziessel, Helvetica Chimica Acta **1986**, 69, 1990-2012

[14] S. Cosnier, A. Deronizer, J.-C. Moutet, J. Mol. Catal.**1988**, 45, 381-391

[15] E.E. Benson, C.P. Kubiak, A.J. Sathrum, J.M. Smieja, Chem.Soc.Rev. 2009, 38, 89-99

[16] E. Barton Cole, P.S. Lakkaraju, D.M. Rampulla, A. J. Morris, E.Abelev. A.B. Bocarsly, J.AM.CHEM. SOC **2010**, 132, 11539-11551

[17] H. Li, P. H. Opgenorth, D. G. Wernick, S. Rogers, T.-Y. Wu, W. Higashide, P. Malati, Y.-X. Huo, K. Myung Cho, J. C. Liao, Science **2012**, 335, 1596

[18] Y.Jiang, M. Su, Y.Zhang, G.Zhan, Y.Tao, D.Li, International Journal of Hydrogen Energy, **2013**, 38, 3497-3502

[19] U. Ruschig, U. Müller, P. Willnow, T. Höpner, Eur. J. Biochem., **1976**, 70, 325-330

[20] S. Kuwabata, R. Tsuda, H. Yoneyama, J. Am. Chem. Soc., **1994**, 116, 5437-5443

[21] M. Aresta, E. Quaranta, R. Libero, C. Dileo, I. Tommasi, Tetrahedron **1998**, 54, 8841-8846

[22] R. Obert, B. C. Dave, J.Am.Chem. Soc. **1999**, 121, 12192-12193

[23] T. Reda, C. M.Plugge, N. J. Abram, J. Hirst, PNAS **2008**, 105, 10654-10658

[24] T.D.H. Bugg , Introduction to Enzyme and Coenzyme Chemistry, 2nd edition

[25] S.-w. Xu, Y. Lu, J. Li, Z.-y. Jiang, H. Wu, Ind. Eng. Chem. Res., **2006**,45, 4567-4573

[26] B.D.Hames, N.M. Hooper, Instant Notes Biochemistry, 2nd edition, **2000**, BIOS Scientific Publishers Limited

[27] D.L.Nelson, M.M.Cox , Lehninger: Principle of Biochemistry 3rd editon

[28] J. Koolman, K.H.Roehm, Color Atlas of Biochemistry, 2nd edition, **2005**, Thieme

[29] D.L.Nelson, M.M.Cox ,Lehninger: Principles of Biochemistry, 5th edition

[30] Ichiro Chibata, Immobilized Enzymes – Research and Development, **1978**

[31] O. Heichal-Segal, S. Rappoport, S. Braun, Biotechnol. **1995,** 13, 113-117

[32] Y. Lu, Z.-Y. Jaing, S.-W. Xu, H. Wu, Ind. Catalysis Today **2006**, 115, 263-268