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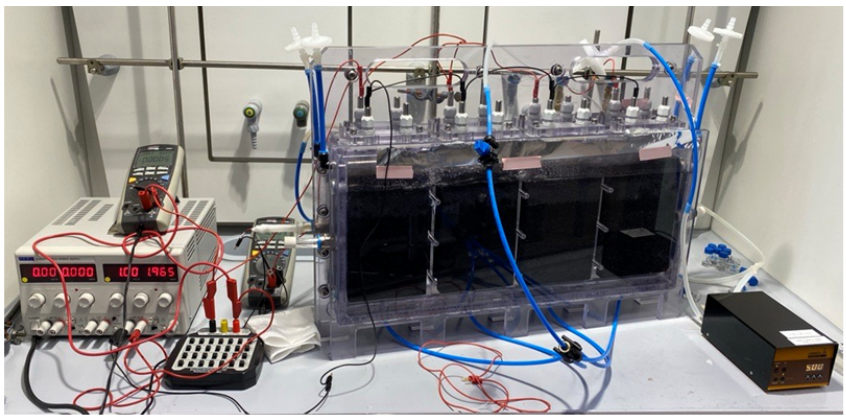
Bundesamt für Energie BFE
Sektion Energieforschung und Cleantech

Final Report 2024

Bioelectric Acetate Synthesis

Executive Summary



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Für den Inhalt und die Schlussfolgerungen sind ausschliesslich die Autoren dieses Berichts verantwortlich.

Inhaltsverzeichnis

Zusammenfassung

Die bioelektrische Synthese von Essigsäure aus CO_2 ist kaum erforscht und ein neuer Weg der zukünftigen industriellen Biokatalyse und Biosynthese. Sie wurde in einem bioelektrochemischen Reaktor mit CO_2-Zufuhr und elektrischer Energie durchgeführt. Eine Methode, die auf mikrobiellen Elektrolysezellen (MEC) basiert. Sie ermöglichte es, die elektrische Energie aus Biomasse und anderen Quellen zu nutzen, um das Überpotential um 0,9 V auf etwa 0,13 V zu senken. Für diese Biokatalyse wurden elektrogene Bakterien verwendet, die zwei CO_2-Moleküle zu Essigsäure reduzieren. Um produktionsrelevante thermochemische und kinetische Bedingungen zu finden, wurde der Protonentransfer über eine Kationenaustauschermembran oder die intermediäre Wasserstoffproduktion untersucht. Die Zukunftsidee ist ein integrierter Prozess, der es ermöglicht, die CO_2-Emissionen aus kommunalen Kläranlagen deutlich zu reduzieren und den Kohlenstoff-Fussabdruck der damit verbundenen Industrien und Haushalte zu verringern. Das Verfahren ist ein Mittel zur Verwirklichung der weithin angestrebten Kreislaufwirtschaft. Aus Essigsäure können anschliessend Kraftstoffe und Chemikalien hergestellt werden.

Résumé

La synthèse bioélectrique de l'acide acétique à partir du CO_2 fait l'objet de peu de recherches et constitue une nouvelle voie pour la biocatalyse et la biosynthèse industrielles futures. Elle a été réalisée dans un réacteur bioélectrochimique alimenté en CO_2 et en énergie électrique. Une méthode basée sur les cellules d'électrolyse microbienne (MEC). Elle a permis d'exploiter l'énergie électrique de la biomasse et d'autres origines pour abaisser le surpotentiel de 0,9 V à environ 0,13 V. Pour cette biocatalyse, des bactéries électrogènes ont été utilisées, qui réduisent deux molécules de CO_2 en acide acétique. Le transfert de protons à travers une membrane échangeuse de cations ou la production intermédiaire d'hydrogène ont été étudiés afin de trouver des conditions thermochimiques et cinétiques pertinentes pour la production. L'idée future est un processus intégré qui permet de réduire considérablement les émissions de CO_2 des stations d'épuration municipales et de réduire l'empreinte carbone des industries et des ménages associés. Le processus est un moyen de réaliser l'économie cyclique largement envisagée. Les carburants et les produits chimiques peuvent être produits ultérieurement à partir de l'acide acétique.

Summary

The bioelectric synthesis of acetic acid from CO_2 is hardly researched and a new pathway of future industrial biocatalysis and biosythesis. It was carried out in a bioelectrochemical reactor with CO_2 supply and electrical energy. A method based on Microbial Electrolysis Cells (MEC). It enabled to harness electrical energy form biomass and other origin to lower the overpotential by 0.9 V to about 0.13 V. For this biocatalysis, electrogenic bacteria were used, which reduce two CO_2 molecules to acetic acid. Proton transfer through cation exchange membrane or intermediate hydrogen production was researched to find production-relevant thermochemical and kinetic conditions. The future idea is an integrated process that allows a significant reduction in CO_2 emissions from municipal wastewater treatment plants and to reduce the carbon footprint of associated industries and households. The process is a means to achieve the widely envisioned cyclical economy. Fuels and chemicals are producible subsequently from acetic acid.

Keywords:

Acetic acid, carbon dioxide, bioelectricity, bioreactive extraction, detergents

1. Introduction

In the context of global warming, carbon dioxide attracts a particular attention. CO_2 is commonly considered as the most abundant greenhouse gas (GHG), which resulting from human activities (burning of fossil fuels). To protect the climate, different international agreements (Paris, Kyoto protocol) have been ratified by the Swiss government in parallel to the national CO_2 act and ordinance. Since the industrial revolution, the anthropogenic production of CO_2 is counter balanced by its absorption by land (26%) and ocean (24%) but approximately, 50% still remains in the atmosphere and accumulates with time (Le Quéré et al., 2009; Canadell et al., 2007).

Another issue is the ever-increasing demand for fossil fuels (oil, coal, gas), because these energy sources are limited on earth. Some estimates predict that oil and gas reserves will run out in 35-37 years, while coal reserves will be exhausted by 2112 (Shafiee et al., 2009). To be prepared for this challenge, it is important to find processes or alternatives to today's carbon sources. One option is to capture CO_2 to produce commodity chemicals. This process is interesting, because it could use CO_2 from the air to produce chemical commodities or biogas. This could perfectly integrate the will to improve the circular economy in Switzerland to mitigate its lack of raw carbon resources.

Today, the world's demand for energy is growing and, despite the advent of renewable energy, the use of fossil fuels is currently unavoidable to meet this demand. The use of this fossil energy results in large amounts of CO_2 being released into the atmosphere and causes environmental disruption. Indeed, between 1990 and 2019, global CO_2 emissions increased by 44% to reach 33.3 Gt of CO_2 produced in 2019 (IEA Status Report, 2019). In Figure 1A, the atmospheric CO_2 concentration constantly increases since decades and reaches a value around 415 ppm in 2021 (US Department of Commerce, 2024). Additionally, the trend in Figure 1B indicates that, between 2010 and 2019, the mean value of the growth rate per year is the highest recorded and confirms the continuous consumption of energy from carbon sources. Even though some efforts are made with the transition to renewable energy sources, the accumulation of CO_2 has already reached a considerable level in the atmosphere. Consequently, the opportunity to mine the raw CO_2 from air seems to be an interesting option as carbon source, especially if this CO_2 can be further transformed to more valuable organic chemicals.

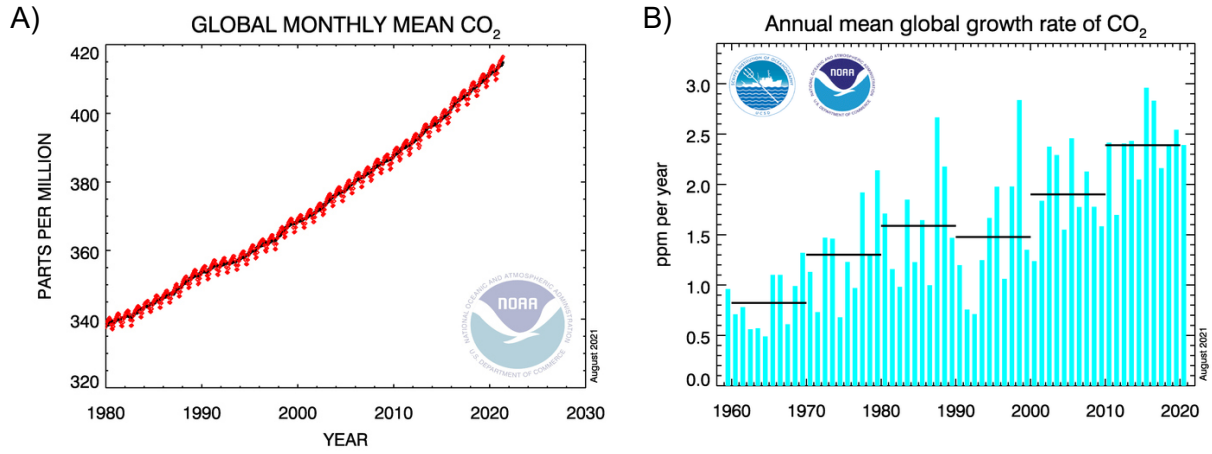

Figure 1: A) Atmospheric CO_2 concentration over the years (1980-now). B) Increase of CO_2 concentration in ppm per year from January to December (1960-now), US Department of Commerce, 2024.

Wastewater treatment plants (WWTPs) produce three different GHGs (CO_2, CH_4 and N_2O) on and off site, and WWTPs are one of the largest secondary sources of GHGs (Corominas et al., 2012; Yerushalmi et al., 2013). An important on-site process at the WWTP in terms of GHG emissions is the aeration of the lagoons to oxidise the organic carbon and release the dissolved GHGs by air stripping (Kyung et al., 2015). Beside the treatment of the wastewater, energy is also needed for sludge digestion (and other unit operations), the maintenance and the transportation of chemicals and fuels for internal consumption (Kyung et al., 2013; Bani Shahabadi et al., 2009). Biogas recovery is an essential energy source for wastewater treatment plants for heating, electricity generation and lagoon aeration. Its use decreases in some way the GHGs emission in WWTP (Bani Shahabadi et al., 2009). Energy efficiency and optimal management of greenhouse gases are still challenges that need to be improved for more sustainable wastewater treatment plants. In the WWT Microbial Fuel Cell (MFC), the oxidation of organic compounds releases CO_2, which could be used to store renewable energy from excess electricity. This idea of reducing CO_2 back into carbon-based fuel or high value-added chemicals with an MFC/WWTP is a possible application for power-to-x technology development.

1.1. Thermodynamics of CO_2 reduction in general

With an oxidation state of +IV, CO_2 is in its highest oxidation state and is the end product of combustion. As a result, CO_2 is thermodynamically very stable and difficult to reduce electrochemically. Figure 2 shows the direct one-electron reduction to the $CO_2^{\bullet-}$ radical. Here as an initial step in the formation of products during electrolysis on inert electrodes (Costentin et al., 2013).

However, this unfavourable process requires a consequent amount of energy to reach the enthalpy of formation of the radical ($CO_2^{\bullet-}$) (Schwarz et al., 1989). The CO_2 reduction potentials decrease significantly when they are coupled with protons and multi-electron transfer. In Figure 2, the decrease of potential for the reduction potential is strongly correlated with the increasing number of electrons involved in the reaction. All these pathways are much more favourable and require much less energy, as indicated by less negative potentials (Barton Cole et al., 2010; Willner et al., 1987).

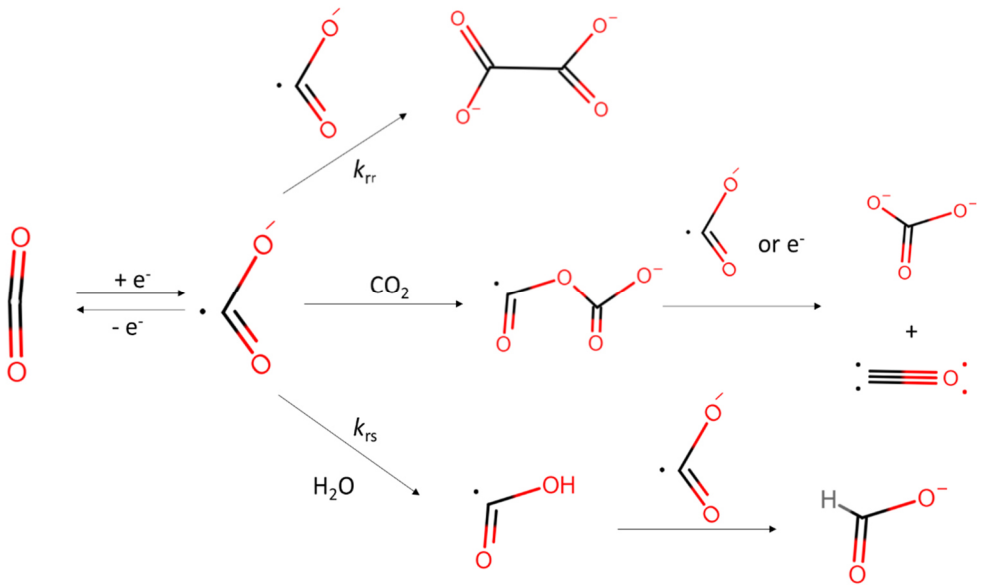

Figure 2: Reduction reaction pathways of CO_2 into oxalate, carbon monoxide and formate on inert electrodes.

However, oxalic acid requires a much more negative potential, probably due to the formation of the carbon-carbon bond, and carbon monoxide, which is below the trend line (Tanaka & Ooyama, 2002).

Although energetically more favourable, proton coupled with multi-electron transfer reduction still requires energy and, more importantly, has to overcome kinetic limitations. The kinetics in electrochemical reactions can be tuned by catalysts, and in parallel, energy drives electrons to the required reaction potential. Several energy sources can be proposed for CO_2 reduction: (i) an electron source can be generated to directly reduce CO_2 if the oxidation potential of the compound is sufficiently negative; (ii) similar to photosynthesis, photocatalytic reductions can produce reduced species of CO_2(Voyame et al., 2016); (iii) an electrode can be used either as a catalytic surface or to transfer electrons at the desired potential to the catalytic species. When considering the electrocatalytic reduction of CO_2 to produce multi-carbon compounds (acetate, ethylene, ethanol), the formation of the C-C bond must be taken into account.

The formation of the C2 molecule (acetate) is seen to be thermodynamically less favourable, requiring a higher potential (-0.8 - -1.0 vs. SHE at pH 7) (Liu et al., 2015).

The principal reason for the supplementary negative potential required for this reaction is the energy necessary for the reaction between two adsorbed species of CO_2, the reduced $-CH_3$ and a radical $CO_2^{\bullet-}$ (Genovese et al., 2017).

A possible pathway for the electrochemical production of acetate is shown to clearly highlight that two molecules of CO_2 need to be adsorbed and react with each other to form the final acetate. Finally, the kinetics of the C-C formation is slower than the hydrogenation of the adsorbed C molecule; this effect limits the rate and selectivity of acetate production compared to the C1 compound (Sun et al., 2017). The main method of tuning the overpotential, rate and selectivity of the acetate production by electrocatalysis is to target the optimized catalyst.

1.2. Microbial electrosynthesis

Microbial electrosynthesis (MES) is a technique, that couples an electrochemical system with electrogenic microorganisms to enable mostly reductive reactions (Rosenbaum et al., 2019). With the suitable microbe, it is possible to convert atmospheric CO_2 into multi-carbon compounds. This makes it possible to convert atmospheric CO_2 into chemical energy and produce value-added compounds (Figure 3). One option is to combine this system with renewable energy, which would allow energy from these sources to be stored (Rosenbaum et al., 2019).

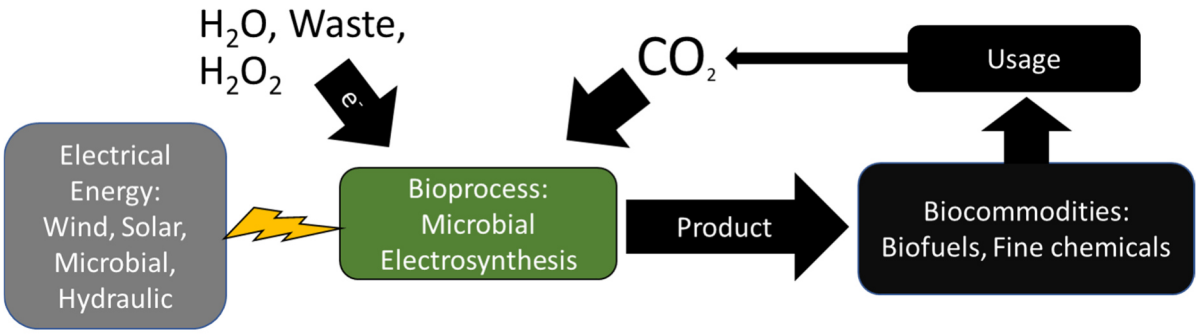

Figure 3: Scheme of the integration of MES in a CO_2 reduction system.

MES is still a recent method aiming at upgrading atmospheric CO_2 to multi-carbon compounds, such as methane or acetate (as in this work), using power and microorganisms to facilitate its reduction.

MES is a relatively new method that is able to generate methane, acetate (as in this work), and a limited number of others using electricity and microorganisms. The main advantage over catalysis with isolated enzymes immobilised on an electrode[22] is that the whole organism allows self-regeneration of the catalysts, greater versatility and more complex metabolic pathways (Rabaey et al., 2010). In contrast, the main drawback is the partial consumption of the substrate for cell growth and maintenance.

Some organisms can transfer to an extracellular solid (like an electrode) or accept electrons. This specific metabolism is called extracellular electron transfer (EET). EET plays a key role in bioelectrochemical systems and can be associated with various applications. The microbial electrosynthesis (MES) employs the EET to work. In this case, the locus of the EET is the cathode and the transfer of electrons is from an electrode to a bacterium. This method of EET is called microbial electron upkeep (EU) (Karthikeyan et al., 2019). Organisms that can do EU are called electrotrophs. They can produce high value-added multi-carbon products by reduction reactions with the consumed electrons. It has been shown that there are two possible pathways of EU during an MES, one direct and one indirect (Karthikeyan et al., 2019).

The indirect pathway with the best-known mechanism is called the H_2-mediated MES and occurs when the dihydrogen is the electron donor for the CO_2 conversion (Figure 4A). The various experiments already carried out show that a potential at the cathode lower than -0.59 V favours the production of hydrogen (Das et al., 2020). This transfer method shows several disadvantages such as (i) the low solubility of H_2 in solution, (ii) the obligation to keep the

system pressurised to have a high concentration of product and, most impacting, (iii) a large amount of electrical energy is required for the proton reduction causing a reduction in energy efficiency. This high demand for electricity is needed to keep the process safe and efficient, because a negative voltage must be maintained at the cathode. To reduce this demand, other electron carriers (redox mediators) can be used such as neutral red (Park et al., 2000), resazurine (Watanabe et al., 2009) or methyl viologen (Gong et al., 2020) (Figure 4A). Another advantage of these electron mediators is their ability to be more soluble than H_2 and therefore can reach larger concentration in solution (Rabaey et al., 2010). The main problem with these transporters is a poor electron transfer ratio that significantly impacts the CO_2 reduction reaction.

The direct pathway for MES occurs when organism directly take electrons from an electrode without the presence of mediators or other electrochemically active species (Figure 4A). This microbial EU allows working at higher potentials at the cathode than in the indirect case thus avoiding high production of dihydrogen. This method is more promising for bio-commodity production as it is more energy efficient (Karthikeyan et al., 2019). The lower electrical energy demand also allows the system to be coupled to a more attractive source of electricity such as an MFC or photovoltaic panel (Nevin et al., 2010). Several classes of microorganisms have already been studied for their ability to perform MES by direct routes such as acetogens, methanogens and photoautotrophic microbes (Tremblay et al., 2015).

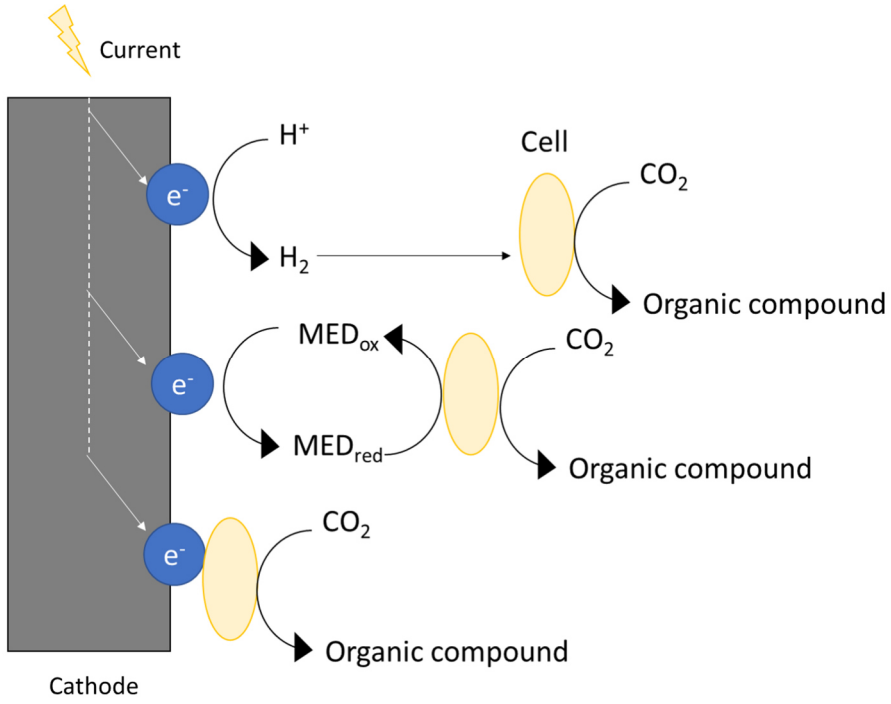

Figure 4: Different mechanisms for the electron transfer in MES.

The process of MES requires an external power supply. This energy causes the anodic reaction to take place, providing the electrons needed to reduce CO_2 in the electrogenic microbes at the cathode. However, MES is complex and presents a number of challenges. The first is the fact that the main substrate is CO_2 which is thermodynamically very stable. Indeed, the reduction of CO_2 is a great challenge in chemistry as explained in Section 1.1. This molecule is stable and inert with a low affinity for electrons and a high energy difference between its occupied and unoccupied orbitals of 13.7 eV (Sun et al., 2017). This means that during electrocatalysis a high overpotential has to be applied to achieve interesting reduction rates, moreover, a low selectivity in the reduction reaction is noticeable (Liu et al., 2017). The other difficulty during MES is the low electron transfer rate between the electrode and the bacteria.

The other major group of organisms that have been extensively studied are the acetogens (Tremblay et al., 2015; Nevin & Lovley, 2011). The production of acetate from CO_2 may be more interesting than that of methane. Indeed, acetate is a compound widely used in the chemical industry. It is also used as a flavour enhancer in the food industry. It can be used as

a carbon source in biotechnological processes. In addition, acetate is a precursor of several chemical compounds. It is also a way to store renewable energy in a more stable and simple way. During an MES, other products may be produced, such as ethanol (Mohanakrishna et al., 2016), butyrate (Nevin et al., 2010) or butanol (Köpke et al., 2009) in small quantities. The ability of these organisms to reduce CO_2 is due to the Wood-Ljungdahl pathway. In acetogens, it is the reduction, which is implied for energy conservation and autotrophic carbon uptake. This pathway and the involved enzyme have been study in *Moorella thermoacetica* (Ragsdale et al., 2008). This metabolic region is divided into two branches, the methyl branch and the carbonyl branch (Figure 5).

The methyl branch consists of the reduction of CO_2 with the help of 6 electrons to make it a methyl group. The carbonyl branch consists of the reduction of CO_2 to carbon monoxide and then undergoes a condensation reaction to form a bond between CO, methyl, and coenzyme A to form acetyl CoA. This latter molecule can then be used to form biomass or be converted to acetyl phosphate to form ATP and acetate (Figure 5).

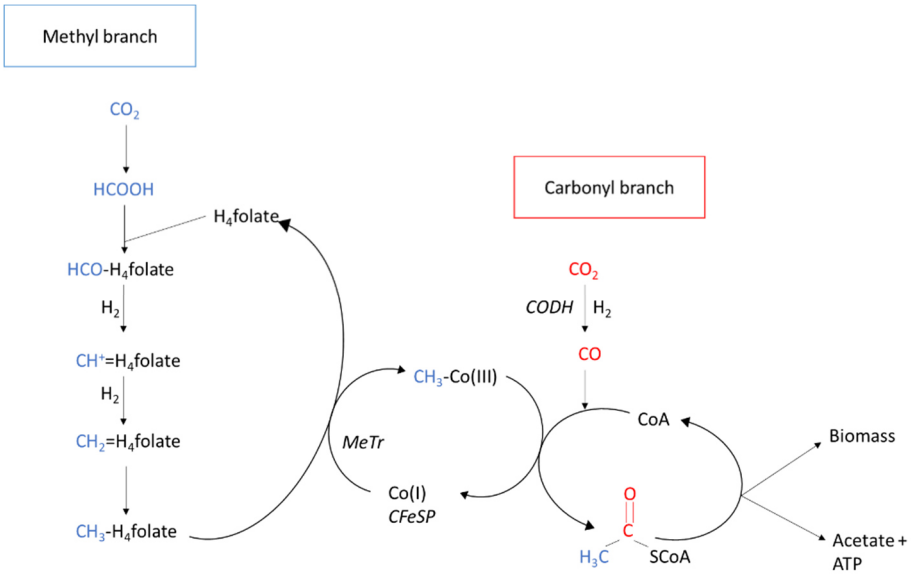

Figure 5: Scheme of the Wood-Ljungdahl pathway.

This metabolic pathway allows CO_2 to be taken up and internalised by the cell before being reduced to acetate. It is also the only autotrophic pathway that can be coupled with energy conservation via ATP formation. The advantages of these microorganisms to produce acetate by electro-biocatalysis is that the process is not limited by the electrochemically formed dihydrogen or formate (methanoate). Indeed, many of these organisms are electrotrophic and

can directly accept electrons at the surface of an electrode (Tremblay et al., 2017). Another advantage is that the electron yield for the production of organic compounds is high, which demonstrates a good efficiency of the Wood-Ljungdahl pathway (Nevin et al., 2010).

Several families of acetogens have already been studied for their use in electro-biocatalysis (Nevin & Lovley, 2011).[35] The *Sporomusa*, *Closridium*, *Acetobacterium* and *Morella* families have already been shown to be electrotrophic and applicable in MES (Das et al., 2020). Many studies on the use of mixed cultures from anaerobic active WWTP sludge or brewery residues have been carried out and have also given encouraging results for the future (Roy et al., 2021; Bajracharya et al., 2017; Song et al., 2019).

Acetate is only the first step towards more interesting compounds for industrial use. Indeed, there are already studies looking into the genetic modification of these electrotrophic organisms that would allow the production of longer and more economically interesting carbon compounds. This is the case of *Clostridium ljungdahli*, which is being studied as a future chassis for various MES productions (Leang et al., 2013) and which, after genetic modification, has already allowed the production of butyrate (Ueki et al., 2014).

The majority of these acetogens are strict anaerobic organisms. The cultivation of anaerobes is a non-negligeable challenge. Most anaerobic organisms are totally intolerant to any exposure to oxygen.

Sporomusa ovata is an anaerobic gram-negative bacterium. This organism is the most studied for MESs in pure culture. Indeed, many studies have already been realized and proved the ability of *S.ovata* to form electroactive biofilms on the surface of electrodes and thus convert 2 CO_2 molecules into acetic acid (Nevin & Lovley, 2011). In all these studies, electron to acetate conversion rates of 80-90% were found, which makes it a promising organism for the future. For this reason, this organism is a reference and is compared to different organisms in studies searching for other acetogens for the MES process (Nevin & Lovley, 2011). Other studies use this organism to verify the effectiveness of other parameters such as electrode coating to optimise the process (Zhang et al., 2013).

The electrobiological process studied in this work is illustrated in Figure 6. The two half-reactions (1.a) and (2) will occur in the MES. The most common anodic reaction (1.b) that provides electrons and protons is water oxidation, which is economically more advantageous than hydrogen peroxide. However, H_2O_2 was preferred in this work, initially, because it allowed

to work at lower and better-defined potentials. Furthermore, as H_2O_2 is the limiting source of protons and electrons, the stoichiometric calculations of the transferred electrons number are relatively simple and depend on the concentration of H_2O_2. Finding that the use of H_2O_2 is equally limiting the process the water oxidation was tired too, which improved results for the moment. The cation exchange membrane separates both sides of the MES and is permeable to cations for the transfer of protons to the cathode where they will be used for CO_2 reduction. Reticulated vitreous carbon (RVC) electrodes were chosen for their inert nature and large specific surface area, which allows a better electron transfer between the electrode and the biofilm.

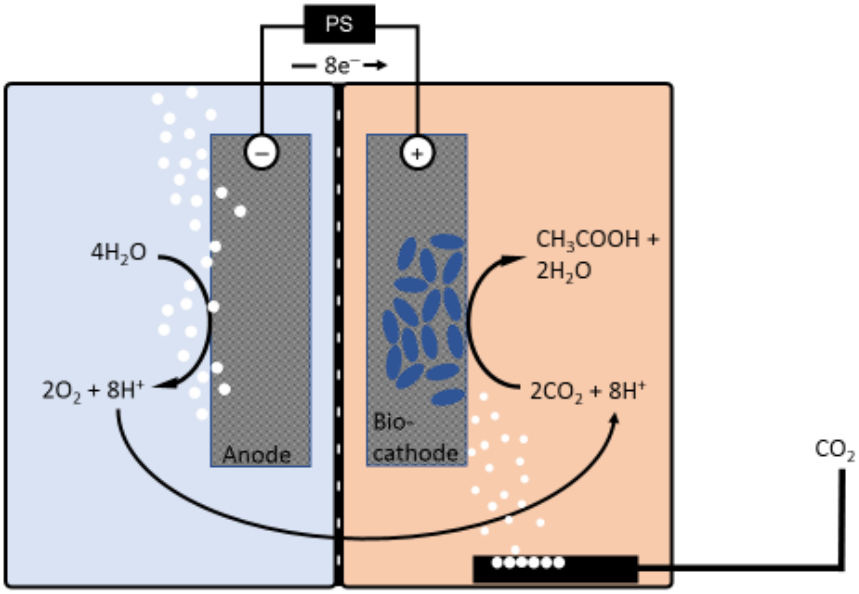

Figure 6: Graphical scheme of the MES operation during the experiment.

Anode: $H_2O_2 \rightarrow O_2 + 2\ H^+ + 2\ e^-$ (0.70 vs SHE, pH = 0) (1.a)

$2\ H_2O \rightarrow O_2 + 4\ H^+ + 4\ e^-$ (1.23 vs SHE, pH = 0) (1.b)

Cathode: $2\ CO_2 + 8\ H^+ + 8\ e^- \rightarrow CH_3COOH + 2\ H_2O$ (2)

1.3. Bioreactive extraction

Nowadays, the majority of acetate production is chemical. Only 10% of production is based on a bioprocess (Vidra et al., 2018). The preference for chemical synthesis is due to the low final acetate concentration obtained by biotransformation, which causes additional difficulties in isolating the acetate from the process medium, which may contain biomass. The recovery of carboxylic acid from aqueous medium is a major challenge in pharmaceutical and fine chemical industry. Various techniques are used to recover the acetic acid produced. Distillation is used with highly concentrated acidic solutions but the energetic demand is high (Saha et al., 2000). Liquid-liquid extraction is limited by phase separation and distribution of the components involved in the reaction system. Another method is the precipitation of carboxylic acid with calcium hydroxide followed by filtration and the recovery with addition of sulfuric acid. Nevertheless, this method seems not to be sustainable economically and environmentally (Eyal et al., 1993).

Reactive extraction is an important field of research for the recovery of carboxylic acid. In this new purification method, one couples enzymatic reactions with extraction techniques. The use of biocatalysts has many advantages, such as improved reaction selectivity and separation, and the ability to recycle the catalyst for many cycles (Datta et al., 2015). In this work, a lipase (Novozyme 435) will be used to catalyse the acetate esterification (with n-butanol). The synthesized ester will be isolated in the organic phase by liquid-liquid extraction and undergoes a hydrolysis with the same Novozyme 435 (N435) to recover purified acetate through an inverse liquid-liquid extraction (

Figure **7**). There are many challenges involved in the development of this process. These include the low concentration of molecules in the aqueous phase to allow complete esterification and release of the acetic acid. The choice of the solvent is a key-step in liquid-liquid extractions. It should be insoluble with the aqueous phase, should have a high affinity for the product of the esterification, and it should not inhibit or denature the N435.

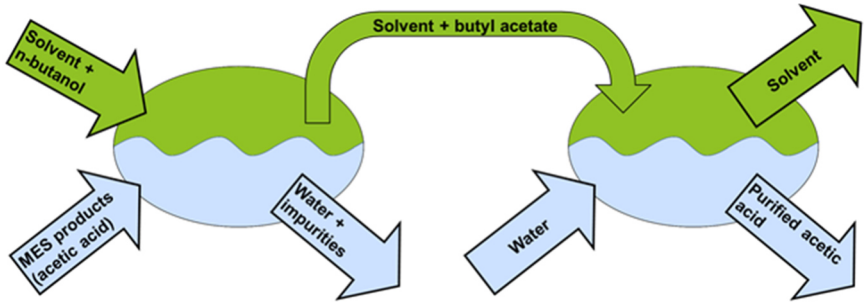

Figure 7: Scheme of the reactive extraction.

1. Objectives

The general goal of the present report is to develop a microbial electrosynthesis (MES) employing a pure cultivation of the anaerobic microorganism *S. ovata*, in order to build a system which can produce acetate having a good coulombic efficiency. The data in the literature showed that in MES different chemicals can be produced using essentially electricity and microorganisms that are able to perform the electron upkeep. In this project research, different challenges had to be afforded to build up a system which could work as a MES to reduce CO_2 to acetate.

- The strain *S. ovata* is considered as a strict anaerobic microorganism and for that reason, a basic methodology for anaerobic cultivation has to be established.
- *S. ovata* will be also tested in a 3 L bioreactor at different temperatures and oxygen levels to understand better the influence of these parameters on the strain growth.
- A MES reactor has to be employed to test the quantity of acetate produced by the strain at different conditions, such as the applied potential. This is important to quantify finally the coulombic efficiency of the system.
- The ability of *S. ovata* to produce an electroactive biofilm has to be tested in a liquid cultivation and MES conditions, where a solid RVC electrode will be employed as support.
- A 16 L-reactor will be also employed to test the acetate production based on the applied potential, in a scale-up condition.
- A methodology for biocatalysis extraction employing a lipase to catalyse acetate esterification will be established in order to improve the extraction yields.

2. Principal results

Several process conditions were investigated to determine the optimal parameters for the microbial electrosynthesis of acetate from CO_2. The main parameters monitored in the fed-batch process were currents, acetate production, and coulombic efficiency at different applied potentials. Different applied potentials resulted in specific coulombic efficiency (CE) and acetate production, respectively.

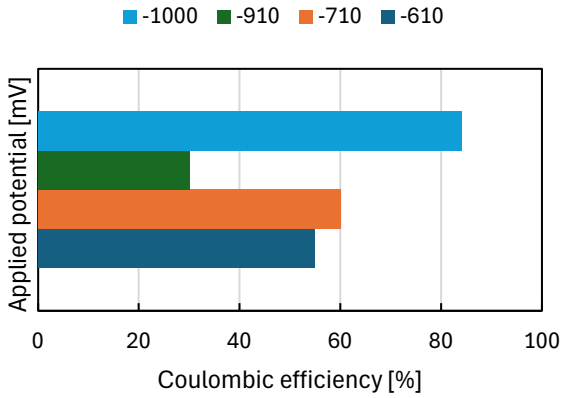

Figure 8: Coulombic efficiency obtained at different applied cathode potentials.

Figure 8 shows the variation of the performance of the MES in response to different applied cathode potentials. When the potential was increased to -1000 mV vs. Ag/AgCl 3 M KCl, the CE showed a remarkable improvement, reaching a value of 84%. In addition, at a lower potential of -610 mV, the CE decreased to a value of 55%. During the various tests conducted, the lowest CE observed was 30%, this showed that the applied voltage is not the only parameter that influences the performance. This type of behaviour has been observed several times, but usually no clear cause was identified. The MES produced a maximum of 15 mmol of acetate and no more, probably due to metabolic inhibition by the acetate produced.

The formation of the S. ovata biofilm was also studied using an electrochemical method, cyclic voltammetry (CV). This method revealed the presence of several peaks specific to the biotic system containing the developed biofilm. Furthermore, the presence of a peak at -500 mV vs. SHE was attributed to the formation of a conductive biofilm capable of producing acetate via CO_2 reduction.

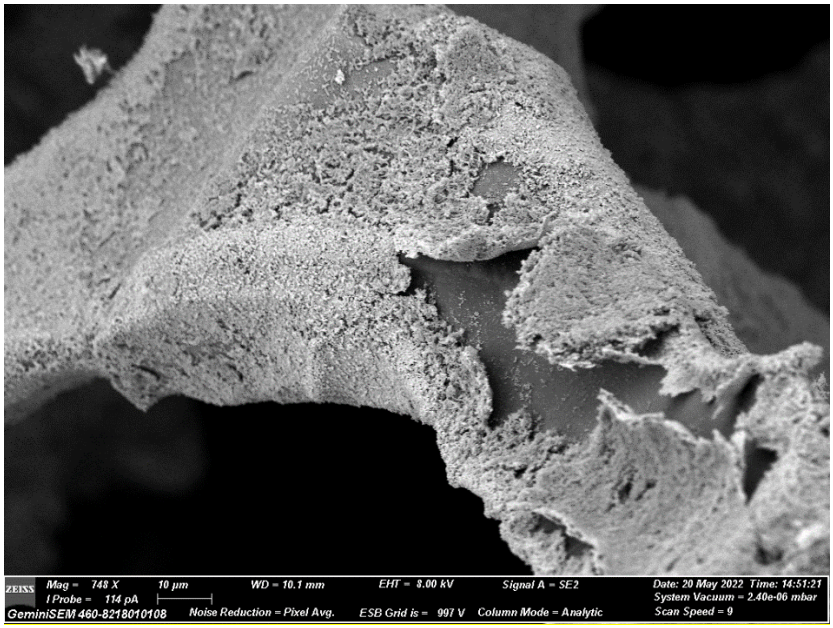

Figure 9: *S. ovata* biofilm after a 3-week MES experiment.

The presence of the biofilm and its proposed morphologic characteristics were confirmed using scanning electron microscopy (SEM) imaging (Figure 9). Electrode samples were analyzed at the end of each MES batch. Figure 9 illustrates the formation of a dense and homogeneous biofilm on the electrode that completely covered the RVC surface.

The recovery of acetic acid remains a significant challenge in such processes, primarily due to the significant dilution of the product and the isolation efforts associated with its extraction. To overcome this problem, a bioreactive reaction was tested to extract acetate from the culture medium. The esterification of acetate and butanol was carried out using lipase N435 in various organic solvents, resulting in the production of butyl acetate (Figure 10).

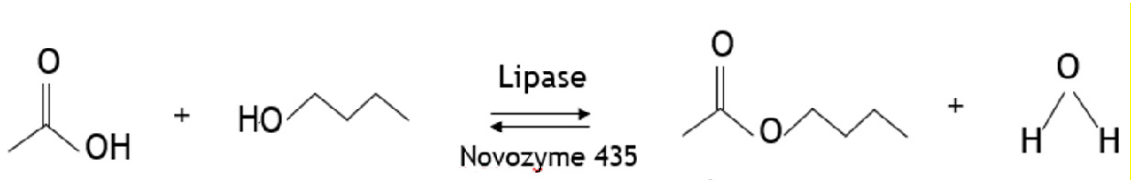

Figure 10: Esterification reaction of acetate with butanol using lipase N435 to produce butyl acetate.

The conversion of acetate to butyl acetate was achieved with a yield of 72% using heptane as solvent. The same method was also used to recover acetate present in a *S. ovata* culture medium, resulting in a conversion of 60%.

3. Future work

Having completed the Research Project, several challenges remain unresolved. The work has repeatedly shown that the applied voltage is only one parameter affecting the conversion of CO_2 to acetate. It remained unclear how much trace oxygen was responsible for the variable acetate production. To better understand the metabolic constraints, it would be beneficial to perform standard bio-transformations where the critical parameters can be assessed. These parameters include oxygen uptake, acetate and CO_2 concentrations. On the theoretical side, it remains to be clarified whether there is a direct electron transfer across the membrane. Once these challenges are overcome, it would be interesting to couple S. ovata biofilm growth on the cathode with corresponding biofilms on the anode using electrogenic microorganisms capable of producing electrons. From this base, further scale-up work can be addressed, which is not yet found in the literature for *Sporomousa ovata* used in this work.

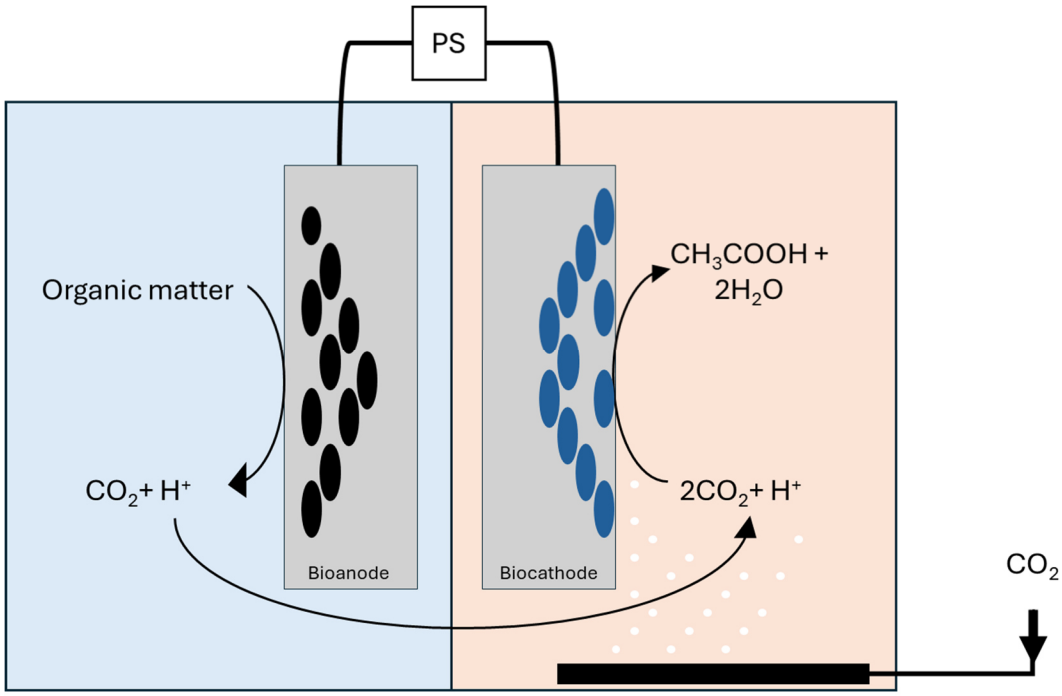

Figure 11: Working principle of an MES connected to a power supply (PS) and containing a biofilm of electrogenic microorganisms at the anode.

A biofilm in the anode compartment would allow the oxidation of organic matter (e.g., present in wastewater or sewage sludge), resulting in the generation of electrons and protons. In addition, carbon dioxide is a by-product whose injection into the cathode compartment may facilitate its reduction by S. ovata, resulting in the production of acetic acid (Figure 11).

4. Take home message

- A microbial electrosynthesis (MES) process was developed using a *S. ovata* biofilm as a cathode. A small-scale two-chamber reactor was used to demonstrate its potential for CO_2 reduction to acetate via electrosynthesis. This challenging approach demonstrated the potential of using MES reactors to convert a very important waste (CO_2) and provide a pathway to useful base chemicals.
- A 16 L MES reactor with the same strain was also tested after acetate and current production with an external applied potential to induce a cathodic current. The results showed on a small scale, and to some extent on a larger scale, that the microbial species used were capable of reducing CO_2 to acetate with low energy input.
- A bioreactive extraction was studied and developed to recover acetate from the culture medium into an aqueous solution. The main advantage was the use of an enzyme to reduce the energy requirements for this downstream process through its selectivity compared to liquid-liquid chemical extractions.

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