Electrons and CO2 to fatty acids through electrolysis and fermentation (EFA)

Ana M. López-Contreras[1], Adriana Rioja Cabanillas[2], Mathieu Ahr[3], Jouke Boorsma[5], Roel Bosschaerts[4], Dirk Develter[4], Amber van Doorn[1], Noel Hoff[2], Mattijs Julsing[1], Gizem Kanat[2], Hendrik-Jan Mensink[3], Mark Renkin[4], Mark Sassenburg[2], Tim Speelman[2], Ruud Veloo[1], Janine Verbokkem[1], Nolan van der Willige[2], Emil Wolbert[1], Truus de Vrije[1]

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Contents

1 Summary and approach of the project

This public report describes globally the results obtained during the project **"Electrons and CO2 to fatty acids through electrolysis and fermentation" (EFA),** belonging to the TSE Onderzoek en Ontwikkeling 2022 program from RVO.
The goal of EFA was to develop an innovative, sustainable and economically competitive process for direct CO_2 utilization using novel electrochemistry technologies developed by TNO and new fermentation routes for fatty acids and microbial protein by Wageningen Food and Biobased Research (Fig 1). Biogenic CO_2-emissions from wastewater treatment plants, represented by AquaMinerals, have been used in this project as potential feedstock for the process, resulting in products that contribute to reduction of greenhouse emissions and replace fossil resources. The intended products were microbial oils, which act as intermediate compounds and offer short-term prospects for use in high-value applications such as personal care products and detergents. In EFA, industrial end-users KLK Oleo and ECOVER are part of the consortium and have tested and validated the products against commercial alternatives. In the long term, the microbial oils and fatty acids could also be used for fuel applications and probably even food products.
Results described in **Chapter 3.1** show that electrochemical formic acid production from CO_2 can meet the purity and operational requirements needed for integration with fermentation processes. The results highlight the promise of a three-compartment electro-chemistry configuration, producing salt-free formic acid with minimized downstream processing.
Developments achieved towards the use of formate as substrate by oil-producing yeast strains are summarised in **Chapter 3.2**. The first steps towards the introduction of a novel pathway for assimilation of formate into biomass and product have been realised. The tolerance to formate and its fermentation potential in combination with glucose or acetate as substrates have been characterised. In addition, a formate stream from the electrochemical process was successfully used as fermentation substrate in a preliminary test.
Fermentation at 100 L scale and subsequent oil isolation have been carried out(**Chapter 3.3)**. The oils produced were sent to KLK Oleo for derivatization to esterquats. Subsequently, ECOVER carried out evaluation tests of the product as an ingredient in textile softener, which showed promising results. The defatted yeast biomass, a side stream from the microbial oil production, showed a protein content of 15% (dry matter) and was further characterized to investigate its potential use as a feed ingredient. This total-use concept will support feasibility of potential applications in the future.
Chapter 3.4 summarizes the results of a preliminary techno-economic assessment towards the feasibility of producing long-chain fatty acids through an integrated two-step CO_2-to-formate-to-fatty-acid conversion process. The analysis, grounded in process flow diagrams and design assumptions derived from ongoing technology development work, demonstrates both the promise of the concept and the substantial challenges that must be addressed before commercial deployment becomes viable.

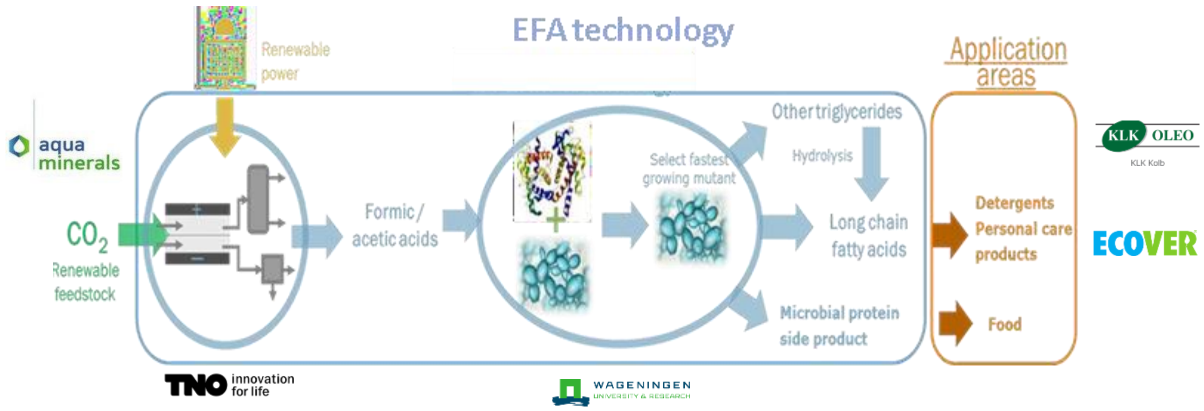

Figure 1 Scheme of the EFA value chain and partners in the project.

2 Samenvatting en projectaanpak

Dit rapport beschrijft een samenvatting van de resultaten die zijn behaald binnen het project **"Electrons and CO_2 to fatty acids through electrolysis and fermentation" (EFA)**, dat deel uitmaakt van het programma **TSE Onderzoek en Ontwikkeling 2022** van RVO.
Het doel van EFA was het ontwikkelen van een innovatief, duurzaam en economisch concurrerend proces voor directe CO_2-benutting, door gebruik te maken van nieuwe elektrochemische technologieën ontwikkeld door TNO en nieuwe fermentatieroutes voor vetzuren en microbiële eiwitten door Wageningen Food & Biobased Research (Fig. 2). Biogene CO_2-emissies afkomstig van afvalwaterzuiveringsinstallaties, vertegenwoordigd door AquaMinerals, zijn binnen dit project ingezet als potentiële grondstof voor het proces. Op deze manier, een reductie van de emissie van broeikasgassen van deze installaties kan gerealiseerd worden, naast een reductie van het gebruik van fossiele grondstoffen voor chemie of andere toepassingen. De beoogde producten zijn microbiële oliën, die fungeren als intermediaire verbindingen en op korte termijn perspectief bieden voor toepassing in hoogwaardige markten, zoals persoonlijke verzorgingsproducten en detergenten. Binnen EFA maken industriële eindgebruikers KLK Oleo en ECOVER deel uit van het consortium; zij hebben de geproduceerde materialen getest en gevalideerd ten opzichte van commerciële alternatieven. Op de langere termijn kunnen de microbiële oliën en vetzuren ook worden ingezet voor toepassingen in brandstoffen en mogelijk zelfs in voedingsmiddelen.
De in **hoofdstuk 3.1** beschreven resultaten laten zien dat elektrochemische productie van mierenzuur uit CO_2 kan voldoen aan de zuiverheids- en operationele eisen die nodig zijn voor integratie met fermentatieprocessen. De resultaten onderstrepen het potentieel van een elektrochemische configuratie met drie compartimenten, waarmee zoutvrij mierenzuur kan worden geproduceerd met minimale nabehandeling. De ontwikkelingen richting de omzetting van formiaat in olieproducerende giststammen zijn samengevat in **hoofdstuk 3.2**. De eerste stappen richting de implementatie van een nieuwe route voor assimilatie van formiaat in biomassa en product zijn gerealiseerd. De tolerantie voor formiaat en het fermentatiepotentieel ervan in combinatie met glucose of acetaat als substraat zijn gekarakteriseerd. Daarnaast is een formiaatstroom afkomstig uit het elektrochemische proces in een eerste proef succesvol gebruikt als substraat voor fermentatie.
De productie van microbiële oliën is op 100L schaal uitgevoerd (**hoofdstuk 3.3)**. De geproduceerde oliën zijn door KLK Oleo gederivatiseerd tot esterquats. Vervolgens heeft ECOVER evaluatietesten uitgevoerd van het product als ingrediënt in wasverzachter voor textiel, waarbij veelbelovende resultaten zijn behaald. De ontvette gistbiomassa, een nevenstroom van de microbiële olieproductie, vertoonde een eiwitgehalte van 15% (op droge-stofbasis) en is verder gekarakteriseerd om het potentieel als veevoercomponent te onderzoeken. Dit concept van volledige benutting (total-use concept) draagt bij aan de haalbaarheid van toekomstige toepassingen.
De resultaten van een eerste techno-economische analyse uitgevoerd om de haalbaarheid te beoordelen van de EFA waardeketen zijn beschreven in **hoofdstuk 3.4**. De analyse, gebaseerd op processtroomschema's en ontwerpaannames die voortkomen uit lopend technologisch ontwikkelingswerk, laat zowel de belofte van het concept zien als de aanzienlijke uitdagingen die moeten worden overwonnen voordat commerciële toepassing haalbaar is.

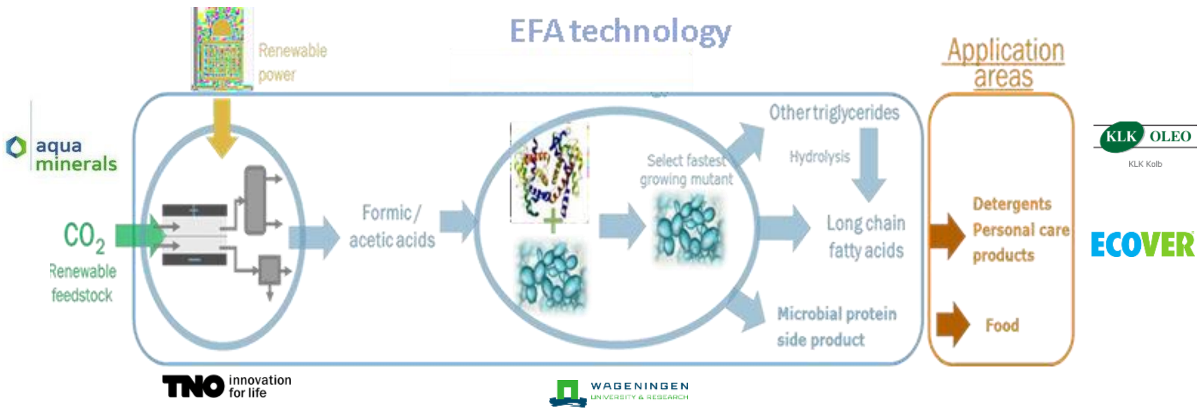

Figure 2 Scheme of the EFA value chain and partners in the project.

3 Results

3.1 Electrochemical production of formic acid in aqueous electrolytes

Electrochemical CO_2 reduction (CO_2RR) provides a pathway to convert carbon dioxide into useful chemical intermediates using electricity, preferably from renewable sources. Within the EFA project, formic acid was selected as an intermediate product because it can be produced with relatively high selectivity and in liquid form, which facilitates storage and transport, and can be directly used as a carbon source for microorganisms in downstream fermentation processes aimed at producing fatty acids.

In the electrochemical system, CO_2 is converted at the cathode through a reduction reaction. CO_2 gas is supplied to the cathode, where it reacts with water and electrons provided by an external power source. This reaction leads to the formation of formate, which can be present as an anion in solution or further converted into formic acid depending on operating conditions and downstream processing. At the anode, a complementary oxidation reaction takes place, typically involving the conversion of water or hydroxide ions into oxygen. These two reactions at the electrodes are physically separated by a membrane but ionically connected through a membrane. By combining the electrochemical reactor with an external power supply continuous operation of the system can be facilitated. A known challenge in conventional liquid-phase electrochemical CO_2 conversion is the limited solubility of CO_2 in water, which restricts the rate at which CO_2 can reach the reactive surface and therefore limits overall performance. In this project, this was addressed by using gas diffusion electrodes (GDEs). These electrodes have a porous structure that allows CO_2 gas to be delivered directly to the catalyst layer, maintaining a high local CO_2 concentration at the reaction interface. As a result, the system can achieve higher reaction rates compared to conventional designs in which CO_2 must first dissolve in the liquid phase. A schematic representation of a GDE is shown in Figure 3.

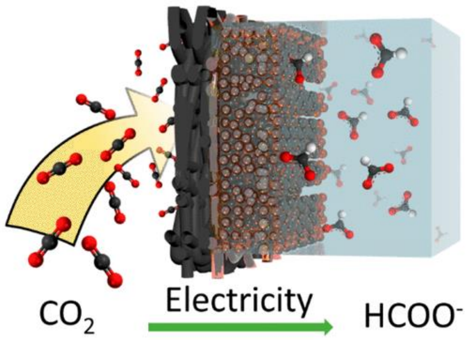

Figure 3 Schematic representation of conversion of CO_2 to formate using a GDE (Grigioni et al. 2021)[1].

To support the development of the electrochemical process, different reactor configurations and laboratory setups were explored and evaluated. Figure 4 shows examples of commercially available electrochemical cells that were used as a reference and starting point for system design and benchmarking. Within the project, these configurations were further adapted to meet the specific requirements of CO_2-to-formate conversion under conditions compatible with downstream fermentation. Figure 5 presents one of the laboratory setups used at TNO, where the electrochemical experiments were carried out and key operating parameters were tested. These activities supported iterative optimisation of the system configuration, contributing to improved understanding of reactor performance and integration within the overall EFA process.

[1] Grigioni, I., Sagar, L. K., Li, Y. C., Lee, G., Yan, Y., Bertens, K., Miao, R. K., Wang, X., Abed, J., Won, D. H., Garc, F. P., Ip, A. H., Sinton, D., & Sargent, E. H. (2021). CO 2 Electroreduction to Formate at a Partial Current Density of 930 mA cm − 2 with InP Colloidal Quantum Dot Derived Catalysts. https://doi.org/10.1021/acsenergylett.0c02165

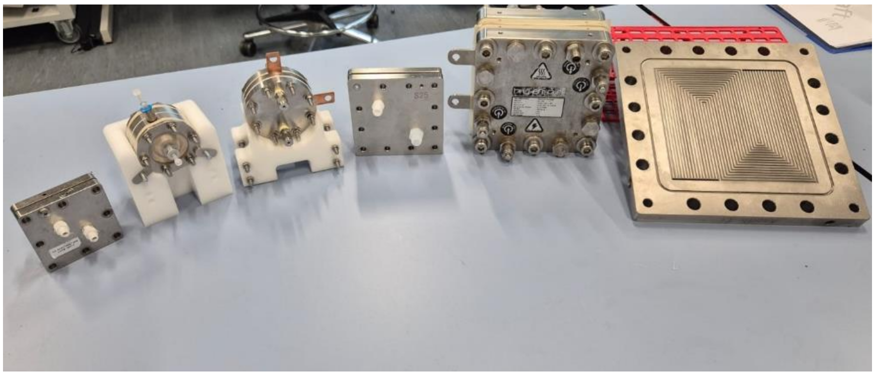

Figure 4 ***Example of commercial electrochemical cells used for CO_2 reduction with Gas Diffusion Electrodes (GDEs). (Source: TNO)***

The work carried out demonstrates that electrochemical production of formate and formic acid is technically feasible under conditions compatible with downstream fermentation. The system can be operated in aqueous environments with relatively low electrolyte concentrations, which is important to ensure compatibility with biological processes. The stability of the electrode materials was assessed, and no release of contaminants was observed under the tested conditions. This is important to ensure that the electrochemical step can be combined with fermentation without negatively affecting the microorganisms.

Overall, the results demonstrate that electrochemical conversion of CO_2 to formate or formic acid can meet the key functional requirements for integration within the EFA concept, providing a solid technical basis for coupling with downstream fermentation. At the same time, the assessment highlights opportunities for further optimisation, particularly in improving energy efficiency, conversion rates, and overall process performance. Addressing these aspects is expected to enhance system performance and support continued progress toward efficient and economically competitive operation at larger scale.

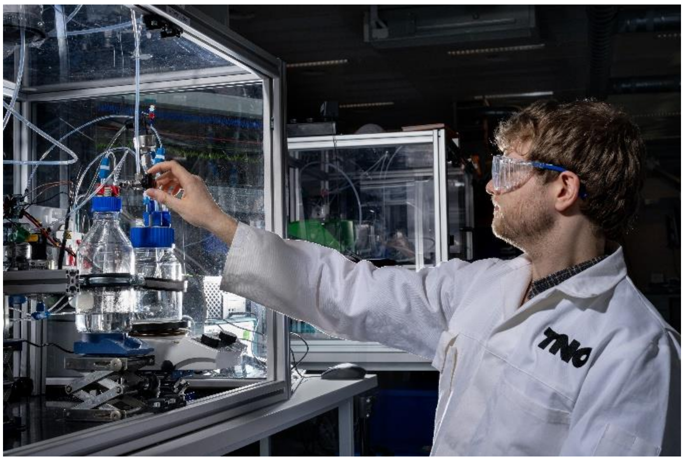

Figure 5 **Laboratory setup used for electrochemical CO_2 conversion tests within EFA at TNO. (Source: TNO)**

3.2 Microbial conversion of formic acid into microbial oils

Formic acid is increasingly recognized as a promising one-carbon (C1) growth substrate for microorganisms. As a readily soluble and transportable molecule, it offers key advantages over gaseous C1 substrates such as CO_2, CO, or methane. Formate produced through reduction of biogenic CO_2 emissions using renewable electricity creates a direct link to sustainable microbial biomanufacturing of biobased products. Many microbial species naturally possess pathways—such as the tetrahydrofolate-dependent C1 assimilation route or the reductive glycine pathway—that enable them to convert formate into central metabolites. Through metabolic engineering, this assimilation can be further optimized and expanded allowing microbes to use formate as both carbon and energy source. This opens the door to novel bioprocesses for the production of fuels, chemicals, and biomaterials with a reduced carbon footprint.

Oleaginous yeasts are able to accumulate up to 50% of their dry biomass as oils, mainly triglycerides comparable to vegetable oils. These oils can form potential alternatives to e.g. palm oil. An advantage of microbial oil production might be the use of cheap feedstocks. In this project we explored the use of formic acid derived from the electrochemical conversion of carbon dioxide as substrate for growth, energy supply, and oil accumulation. Lian *et al* (2012)[2] reported on the beneficial effect of formic and acetic acids on oil accumulation in *C. oleaginosus, L. starkeyi and R. glutinis.* These organisms used additional energy supply from formic acid while growing on acetic acid. In EFA, we investigated this observation in *C. oleaginosus* in detail using different media. To extend the use of formic acid towards fermentation products or biomass, a strategy was developed to implement a new metabolic pathway for formic acid fixation. The first steps of this route were achieved during the project, including the generation of a genetically engineered strain that shows modified amino acid metabolism.

The oleaginous yeast *C. oleaginosus* grew well and tolerated up to 25 g/L of formate (added as formic acid) in the medium **(Fig 6)**. However, in contrast to the results in Lian *et al* (2012), formate co-utilisation did not result in higher yields of oils under the conditions tested. The medium composition, and specifically a high C/N ratio is important to obtain high yields of oils. Metabolic engineering of oleaginous yeasts to enable formate conversion as carbon source is a promising route towards success. Initial steps, i.e. the introduction of knock-outs to improve formate metabolism were successful, but require more attention to establish feasible fermentation processes.

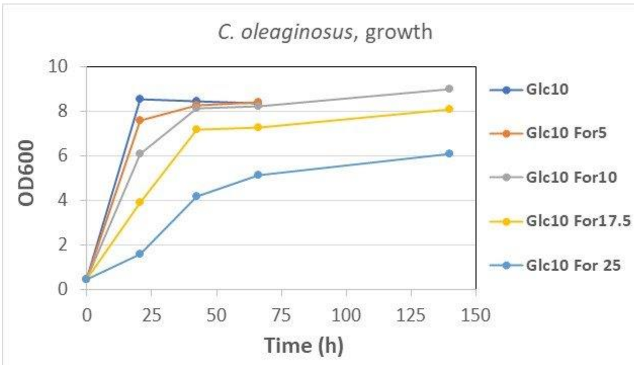

Figure 6. Growth of oleaginous yeast on glucose (10 g/L) supplemented with formic acid at 5, 10, 17,5 and 25 g/L.

In a parallel approach, a formate-containing stream from electro-chemical CO_2 conversion by TNO was used as a co-substrate in a fermentation with a control bacteria strain. The formate in the stream was successfully utilized, indicating that this stream could potentially be included as co-substrate in a fermentation process.

Microbial oils have been produced in a fermentation at 100-L scale to provide samples for application testing. Different kinds of oils have been produced by oleaginous species growing on a glucose substrate. These samples were representative of oils produced on an organic substrate. In Figure 7, the bioreactor used for the fermentation and the products obtained after oil extraction are shown.

[2] Lian, J. et al. "Yeast fermentation of carboxylic acids obtained from pyrolytic aqueous phases for lipid production". In: *Bioresource technology* 118 (2012) p.177-186. doi: 10.1016/J.BIORTECH.2012.05.010. url: https://pubmed.ncbi.nlm.nih.gov/22705522/

Figure 7 ***Bioreactor used for fermentation, microorganism and products obtained: oils and defatted biomass. (source: WFBR)***

3.3 Application testing

Microbial oils to textile softener

The produced microbial oil consists of a mixture of free fatty acids, acylglycerides, phospholipids and other components (Zainuddin *et al*, 2021)[3]. The triglycerides were hydrolysed at high temperatures to produce free FAs and glycerol via the reaction in Figure 8, then distilled at low pressure to remove colour components. A Sample of distilled fatty acid mixture was obtained, with acid and saponification values of ca. 200 mg KOH/g, respectively, and an iodine value of 63 g I_2/100g material.

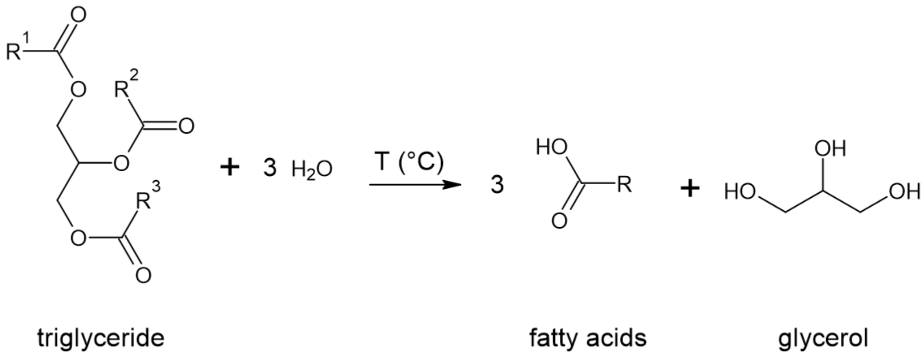

Figure 8 ***Fatty acid synthesis from triglycerides in microbial oil. Adapted from KLK OLEO***

The microbial oil samples produced in the project were chemically characterised, and one of the batches was sent to KLK Oleo for derivatization to a chemical product with applications in cleaning products. This product, an esterquat, was used by Ecover in a test as softener for textile products and compared to one of the current products used in their process. The performance of the new esterquat was very similar to the

[3] Zainuddin MF et al. Current Pretreatment/Cell Disruption and Extraction Methods Used to Improve Intracellular Lipid Recovery from Oleaginous Yeasts. *Microorganisms*. 2021; 9(2):251. https://doi.org/10.3390/microorganisms9020251

performance of the reference tests carried out under standard conditions for tests of textile softeners, which is a promising result for future applications of microbial oils

Defatted yeast material characterization

The yeast biomass remaining after oil extraction, the “defatted yeast”, was analysed for content in carbohydrates, protein and other potentially interesting components. The defatted biomass still contained remaining oils, at approx. 10 % of the dry matter (Table 1). These oils showed the same composition as those extracted. Major components in this material were sugars, mostly glucose, accounting for 37.4% of the dry matter, corresponding mainly to cell wall polymeric components and protein (15% of the dry matter). The biomass contained essential amino acids needed in animal diets, constituting a potential ingredient for feed applications.

Table 1 Composition of defatted yeast material (yeast biomass after extraction of fatty acids).

		(% of dry matter)					
Sample	**Dry matter (g/100g sample)**	**Protein (Kjeldahl)**	**Ash (550°C)**	**Aminoacids (18)**	**Oil/ lipids (extracted)**	**Neutral sugars**	**Uronic acids**
Defatted yeast material	81.8	15	5.9	9.7	10.4	37.4	0.8

The defatted biomass contained approx. 6 % (dry matter)) of ashes (Table 1), which indicate salts and other elements. This ash content is similar to that reported by Jia et al (2023)[4] for typical feed for poultry (5.7 % weight of crude sample), swine (6.2 % weight of crude sample) and fish (8.7 % weight of crude sample).

3.4 Techno-economic analysis

In EFA, a techno-economic assessment was conducted to evaluate the production of the target molecules via an integrated two-step conversion pathway. This process combines electrochemical conversion of CO_2 with a subsequent fermentation process. The objective of the assessment was to identify key process parameters and boundary conditions required for economically viable production. The results provide insight into the current state of development of the technology and identify the main challenges that must be addressed to enable future scale-up and implementation.

[4] Jia, Z., Zhou, J., Yang, M., Wang, M., Li, L., & Fan, X. (2023). Preparation and evaluation of certified reference materials for crude protein, crude fat, and crude ash in feed. Microchemical Journal, 191, 108854. https://doi.org/10.1016/j.microc.2023.108854

***Figure 9** Installation run by the Water Authority The Dommel in Tilburg, where liquid CO_2 and biomethane are produced. (Source: Aquaminerals)*

The overall process for the conversion of CO_2 into fatty acids is presented schematically in Figure 10. In the first step, residual gases resulting from the anaerobic digestion of sewage sludge from a wastewater treatment plant are captured. The CO_2 is separated from the other gases (mostly methane) after an upgrading treatment. An example of a current installation producing liquid CO_2 and biomethane is shown in Fig 9. The purified CO_2 is subsequently fed to an electrolyser, where it is converted into either formate or formic acid, depending on the system configuration. In the case of formate production, an additional bipolar membrane electrodialysis (EDBPM) step is required to acidify the stream to formic acid. During fermentation, the formic acid is biologically converted into long-chain fatty acids under aerobic conditions. To improve overall carbon efficiency, unconverted CO_2 from the electrochemical conversion step and CO_2 present in the fermentation off-gas are recovered via liquefaction and recycled within the process. In the final step, the fermentation broth is subjected to solid–liquid separation, drying, and mechanical pressing to remove water and isolate the fatty acid product.

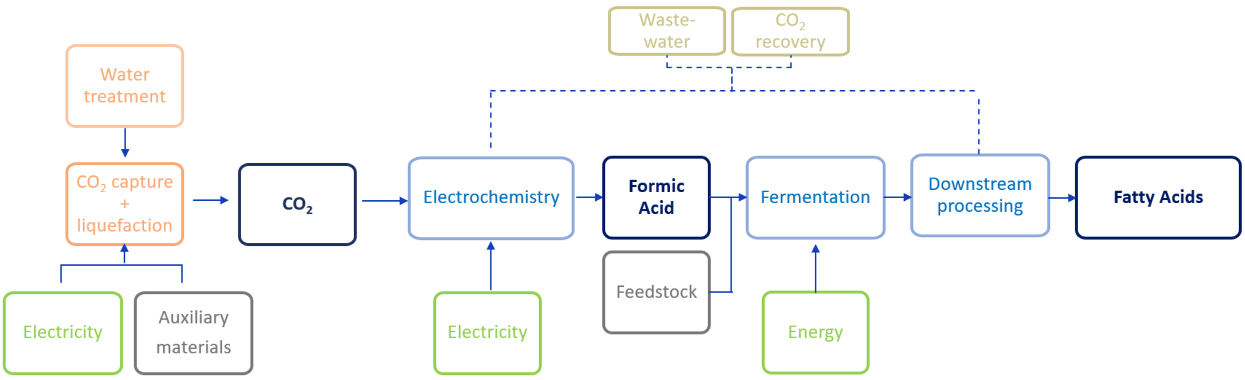

***Figure 10** Schematic value chain for EFA process*

The study provides valuable insights into key areas for further optimisation toward commercial deployment. First, the process is characterised by a relatively high electricity demand, primarily due to the electrochemical conversion step and the bipolar membrane electrodialysis unit. As a result, electricity consumption is a major contributor to operating costs, with current production cost estimates exceeding market prices for fatty acids. In addition, the carbon conversion efficiency in the fermentation step presents opportunities for improvement. Currently, approximately 20% of the carbon in the formic acid feed is incorporated into the fatty acid product, while a substantial fraction is released as CO_2. Enhancing carbon utilisation would further strengthen overall process efficiency and reduce both energy demand and feedstock requirements.

The assessment indicates that improvements in carbon conversion efficiency could substantially enhance the economic performance of the process. Increasing the carbon yield to a range of 30–50% would reduce substrate consumption and decrease the specific energy requirement per tonne of product. In combination with reductions in electricity demand in the electrochemical steps, this would bring the process closer to cost levels associated with conventional fatty acid production.

Overall, the techno-economic assessment offers a solid foundation for continued development of the technology. The insights obtained contribute to identifying effective optimisation strategies and support progress toward more efficient and economically viable CO_2-based production routes, in line with future scale-up and implementation objectives.

4 Contribution of EFA to the goals of the call and knowledge position

This projects contributes to MMIP 8: "Maximale elektrificatie en radicaal vernieuwde processen" (Table 2) since the main objective of the project is to produce molecules with high added value via electrochemical utilization of carbon dioxide. Specifically, the project contributes to the development of new technology that uses intermittent renewable electricity directly for electrochemical conversion of biogenic CO_2 into formic acid/formate. In this way, reducing greenhouse gas emissions and enabling production of protein, fatty acids and oils from non-fossil resources. Regarding both oil and protein production, the traditional competitor of the EFA technology is the production of vegetable oils from agricultural crops that requires land, water and use of fertilizers.

In this way, the radically new **EFA process contributes to electrification of the chemical industry,** not only reducing CO_2 emissions but also enabling their utilization to produce **target products that are circular** and have a low carbon footprint.

EFA goals align with the Mission C goals of the 'TKI Energy and Industry Plan'8 on several aspects:

- *The 50% decreased use of primary feedstock*: in the EFA value chain, the source of CO_2 is non-fossil-based.

- *Electrification and CO/CO_2 reutilization*: in the EFA process, CO_2 is valorized though an electrochemical process

- *Radically new process*: the EFA project combines two novel technologies: CO_2 electrolysis and fermentation to create a new process for chemical production

Table 2 Sub-programs in MMIP8 and EFA contributions

Sub programs within MMIP8	*EFA process contribution*
Hydrogen, molecules and renewable fuels (with the exception of renewable fuels for aviation and ships)	EFA technology leads to the sustainable production of products categorized into several classes that can be valorized in various routes: small carboxylic acid (e.g. formic acid, oxalic acid, acetic acid), fatty acids, oils, proteins.
Flexibility and digitalization	EFA is based on the use of renewable electricity as main energy source, especially in the first part of the process.
Radical new processes	EFA proposes a radically new process to efficiently valorize CO_2. The process integrates CO_2 electrolysis with the microbial synthesis of products.
Societal implications of industrial electrification	EFA technology proposes an alternative route to agricultural production of oils and proteins by using CO_2, electricity and microbes. The technology can play an important role in the future production of food and personal care products, but can also be adapted as an alternative to current biofuel production processes.

5 Publications and Spin Offs

Oral Presentation by A. Rioja Cabanillas (TNO). 'Electrochemical CO_2 Reduction to Formate Using GDEs and Its Integration with Fermentation for the Production of Fatty Acids' at the European Materials Research Society Symposium Exploring emerging photo and electrochemical systems for CO_2 conversion to fuels and chemicals. Warsaw, September 2025.

Attendance of Mattijs Julsing to the TKI Energie en Industrie event Projecten op het podium, 16 September 2025, Metaal Kathedraal, Utrecht.

Attendance of Jacco van Haveren to the Carbon Removal (CCUS) program event from RVO, Amersfoort, December 2025.

Project website: https://research.wur.nl/en/projects/electrons-and-co2-to-fatty-acids-through-electrolysis-and-ferment/

"Engineering and evaluation of formic acid utilization for microbial oil production", MSc Internship Report, Amber van Doorn, September 2025 - March 2026. Supervisors Ana M. López-Contreras and Mattijs Julsing (WFBR) and Nico Claassens (WU).

This report is available at: https://doi.org/10.18174/718406

Visits of the EFA team to the Electrochmistry pilot plant at TNO Rijswijk (left, source: TNO) and the KLK Oleo facilities (right, source: WFBR).

6 Contact person and more information on the project

Project coordinator: Ana Lopez Contreras, email: ana.lopez-contreras@wur.nl

Project website: https://research.wur.nl/en/projects/electrons-and-co2-to-fatty-acids-through-electrolysis-and-ferment/

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To explore the potential of nature to improve the quality of life

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