Bio-Electrocatalytic Application of Microorganisms for Carbon Dioxide Reduction to Methane

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Introduction

CO₂ reduction has gained high interest in the last decade because of research in the field of carbon capture and utilization (CCU), which is a viable strategy for cyclic carbon use. From a chemical point of view, CO₂ is a valuable feedstock for many reactions and products such as acids, alcohols, and gases, that is, acetic acid, methanol, and methane. CO₂ is a very stable molecule, therefore, its reduction reactions require a high energy input because of overpotentials and multielectron reduction steps.

According to the standard redox potentials shown in Equations (1)–(6), the reduction of CO₂ can be tuned toward different products. Indeed, the given potentials are much more negative in reality because of overpotentials. To lower these energy barriers, several electrochemical and biological systems have been investigated to catalyze the reduction of CO₂. Particularly, the biological pathway of CO₂ reduction, which uses microorganisms or enzymes, offers a biocompatible and sustainable energy storage approach. This is particularly attractive for industry because of the use of mild reaction conditions such as ambient temperature and pressure. Furthermore, bio-catalysts are capable of self-regeneration and are, therefore, highly suitable for long-term performance systems without the loss of catalyst.[1–4]

We present a study on a microbial electrolysis cell with methanogenic microorganisms adapted to reduce CO₂ to CH₄ with the direct injection of electrons and without the artificial addition of H₂ or an additional carbon source except gaseous CO₂. This is a new approach in comparison to previous work in which both bicarbonate and gaseous CO₂ served as the carbon source. The methanogens used are known to perform well in anaerobic reactors and metabolize H₂ and CO₂ to CH₄ and water. This study shows the biofilm formation of those microorganisms on a carbon felt electrode and the long-term performance for CO₂ reduction to CH₄ using direct electrochemical reduction. CO₂ reduction is performed simply by electron uptake with gaseous CO₂ as the sole carbon source in a defined medium. This “electrometabolism” in such microbial electrolysis cells depends strongly on the potential applied as well as on the environmental conditions. We investigated the performance using different adaption mechanisms and a constant potential of −700 mV vs. Ag/AgCl for CH₄ generation at 30–35 °C. The experiments were performed by using two-compartment electrochemical cells. Production rates with Faradaic efficiencies of around 22% were observed.

In particular, reduction reactions toward alcohols, aldehydes, and other hydrocarbons are of high interest as most of those substances can be applied directly as fuels. If we consider bio-catalysts for the reduction of CO₂, dehydrogenase enzymes are prime candidates. In 1976, Ruschig et al. reported the application of formate dehydrogenase for the reduction of CO₂ to formate with the aid of the coenzyme NADH.[5] Further investigations on the carboxylation of CO₂ using dehydrogenase enzymes have been presented by Aresta and Dibenedetto.[6]
kanth and co-workers used a bio-electrochemical system (BES) with formate dehydrogenase and NADH as a cofactor for the reduction of CO\(_2\) to formate and further presented a detailed study on the electrochemical application of enzymes for the generation of, for example, fuels and chemicals.\[7, 8\] Reda et al. showed a bio-electro catalytic approach with the electrochemical reduction of CO\(_2\) to formate assisted by a formate dehydrogenase (F\(_{DH}\)) enzyme without a sacrificial coenzyme.\[9\] Recently, our group has shown that electrodes with such enzymes immobilized onto it can be used efficiently for the electrocatalytic generation of higher alcohols such as butanol and the reduction of CO\(_2\) to methanol.\[10, 11\] These nonliving biocatalysts, however, are each single molecules that are isolated from corresponding microorganism strains.

In a different approach, the direct application of the living biocatalysts or microorganisms, respectively, was investigated. In particular, living microorganisms have gained interest in comparison to enzymes as their products are tunable by the choice of microorganism strains and environmental parameters. Desired products can be generated with high yield and selectivity after the adaptation is complete.

Several studies have been presented for the application of microorganisms in the field of CO\(_2\) conversion, biosynthesis, and the production of biofuels with H\(_2\) equivalents added artificially. In the 1980s, Kerby and Zeikus, and Sharak Gentner and Bryant presented the growth of different microorganisms utilizing CO\(_2\) as the carbon source among others.\[12, 13\] Tanner et al. showed the generation of acetate by growing Clostridium jungdahlii with, for example, CO, H\(_2\), or CO\(_2\).\[14\] Sakai et al. presented work on ethanol and acetate generation from Moorella sp. and further investigated the influence of the pH and the activities of the corresponding dehydrogenase enzymes.\[15, 16\] Liou et al. presented work on Clostridium strains for the generation of, for example, acetate, ethanol, and higher alcohols such as butanol from CO, CO\(_2\), and others, such as sugars, as the growth support and carbon source.\[17\] Logan further discussed the application of microorganisms in an electrochemical system to establish microbial fuel cells.\[18\] Logan and co-workers screened studies with a focus on microbial electrosynthesis.\[19\] Additionally, Kundiyan et al. investigated Clostridium ragasdalei for ethanol production and the influence of different parameters such as pH and temperature on their performance during fermentation.\[20\] Tracy et al. depicted detailed pathways with all of the corresponding enzymatic steps in reactions of Clostridia. They showed the large variety of possible chemical products and biofuels that can be obtained by using these biocatalysts starting from CO\(_2\).\[21\]

Indeed all of these studies showed the properties and potential to generate valuable carbon-based products using microorganisms. However, most of the approaches that aimed towards CO\(_2\) conversion required fermentation processes and basically focused on the metabolism of the microorganisms to generate fuels and chemicals. In our work we wanted to investigate the possibility of direct electrochemical reduction of CO\(_2\) using such living microorganisms grown on an electrode as biocatalyst. This offers the possibility to tune the metabolisms of the microorganisms toward a certain product, according to the potential applied, and therefore, to increase the selectivity. Furthermore, this approach depicts a method that opens ways for renewable energy storage as solar or wind energy could serve as electrical sources. Therefore, the approach presented here introduces direct electron injection into microorganisms and a charge transfer mechanism for CO\(_2\) reduction.

In contrast to molecular catalysts, for example, metal-organic compounds or enzymes, in which charge transfer occurs through conjugated bonds and metal ions, charge transfer in living systems, such as microorganisms, may occur outside of the cell of the living biological systems through the outer membrane. For microorganisms, it is proposed that bio-electrochemical reactions occur mainly because of so-called extracellular electron transfers with or without the aid of an electron shuttle: Rosenbaum et al.\[22\] suggested three different cathodic extracellular electron transfer mechanisms for biocathodic microorganisms. In addition to a direct electron transfer that involves c-type cytochrome electron transfer chains, they propose a mediated electron transfer to a periplasmic hydro- gensase or a direct electron transfer that involves cytochrome–hydrogenase partnerships. Furthermore, Villano et al. discussed the influence of abiotic hydrogen generation on indirect extracellular electron transfer, which is also considered as a possible pathway for microbial cathodic reactions.\[23\] Ajo-Franklin et al. investigated charge transfer between living and nonliving organisms and tried to apply nanostructures for improved charge transport through cell membranes.\[24, 25\] Bio-electrocatalytic species such as microorganisms, which are capable of direct charge transfer, therefore, have gained interest for applications in electrochemical CO\(_2\) reduction. This work focuses on the conversion of CO\(_2\) to CH\(_4\) using hydrogenotrophic methanogens in a microbial electrolysis cell. The proposed mechanism for methanogenic mixed cultures is correlated to the well-known mechanisms of the conversion of CO\(_2\) and H\(_2\) to CH\(_4\) and water in anaerobic digesters. Deppenmeier et al., Shima et al., and Ferry have discussed not only the detailed enzymological pathways, which include oxidation and reduction reactions for electron transfer within the metabolism, but also the role of metabolic groups for CH\(_4\) production from biomass with such methanogenic mixed cultures.\[26–28\] The metabolic pathways of these methanogens for the conversion of CO\(_2\) to CH\(_4\) can be summarized in the following overall reaction equation (Eq. (7)).

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}
\]  \hspace{1cm} (7)

However, H\(_2\) added artificially, which is generated before CH\(_4\) synthesis, makes these processes unfavorable because of the high cost and energy loss from the storage and transport of H\(_2\). As a different approach, the direct electrochemical reduction of CO\(_2\) using microorganisms as biocatalysts without the need for H\(_2\) added artificially is desired. This would offer direct CO\(_2\) reduction on a biocathode without the need for any mediator or supplementary process such as water splitting for H\(_2\) generation and enable the storage of renewable energies in the form of fuels and chemicals. The first study on CH\(_4\) synthesized bio-electrochemically from CO\(_2\) without any electron
studied the performance of a CH$_4$-producing microbial electrolysis cell (MEC) for 188 days. The maximum energy efficiency obtained in this study was 51.3% in a yield test. Villan et al. presented high CH$_4$ production rates by using a microbial biocathode based on a hydrogencophilic methanogenic culture.\textsuperscript{[23]} In addition, they showed the possibility to establish biofilm reactors and the use of a CH$_4$-producing MEC for wastewater treatment. A bioanode able to oxidize acetate and a CH$_4$-producing biocathode were used in these studies. High acetate removal from the influent and efficient conversion to CH$_4$ was shown, and 75% of the energy was captured in the resulting CH$_4$ gas.\textsuperscript{[32,33]} Moreover, Sato and co-workers discussed the possible implementation of the bi-electrochemical conversion of CO$_2$ to CH$_4$ for geological storage reservoirs. Electromethanogenic CO$_2$ reduction can be achieved by using biocathodes based on subsurface methanogens.\textsuperscript{[36–37]} Furthermore, Li et al. presented the utilization of electromicrobial systems for CO$_2$ reduction and showed the conversion of CO$_2$ to higher alcohols by using genetically modified \textit{Ralstonia eutropha} H16.\textsuperscript{[38]} Recently, Jiang et al. showed a bio-electrochemical approach that used a methanogenic mixed culture for the simultaneous production of CH$_4$ and CH$_3$COOH from CO$_2$. The CO$_2$-rich medium and gaseous CO$_2$ acted as carbon-based nutrients. They adapted microorganisms to a carbon-nutrient-only metabolism by reducing the amount of H$_2$ added stepwise in four cycles of 10 days each.\textsuperscript{[39]}

A more recent study on microbial electrolysis has been presented by Bajracharya et al. who used pure and mixed cultures for CO$_2$ reduction. In their investigations, they applied an assembly of graphite felt and stainless steel as the cathode for the generation of acetate and CH$_4$ from the conversion of bicarbonate.\textsuperscript{[40]} However, all of those approaches were performed using carbonate salts dissolved in the medium as the carbon source in place of or in addition to gaseous CO$_2$. In contrast, Bajracharya et al. also presented a study on a gas diffusion biocathode to provide CO$_2$ directly.\textsuperscript{[41]}

In this work we were interested in using CO$_2$ in its gaseous form for reduction to CH$_4$. To get an idea of the bio-electrocatalytic process, we also investigated the system in a state without any CO$_2$ or bicarbonate but under inert conditions. Here we show a similar approach to the work of Jiang et al.\textsuperscript{[39]} on direct electron injection into methanogenic mixed cultures and the reduction of CO$_2$ to CH$_4$ (Eq. (8)).

\[
\text{CO}_2 + 8 \, \text{e}^- + 8 \, \text{H}^+ \rightarrow \text{CH}_4 + 2 \, \text{H}_2\text{O}
\] (8)

However, in contrast to previous studies, we did not add CO$_2^-$ and used gaseous CO$_2$ only as the carbon source. This was done to preserve the possibility for investigations without any CO$_2$ in the system but under N$_2$-saturated and, therefore, inert conditions. Furthermore, this provides a controlled supply of the carbon source and, therefore, the exact determination of the efficiency and electrochemical characterization of the system for CO$_2$ reduction. These investigations proved that CH$_4$ was only generated if CO$_2$ was added. The approach presented here shows the conversion of CO$_2$, added by purging the gas directly through the system, to CH$_4$ without any other additives required. This is favorable as H$_2$, an explosive gas obtained from energy-costly processes such as the steam reforming of fossil fuels or water electrolysis, can be avoided.

Results and Discussion

After the inoculation of the microorganism suspension (Figure 1a), a constant potential of $-700$ mV vs. Ag/AgCl was applied, and the cathode compartment was purged for approximately 5 h per day with CO$_2$ and H$_2$. The negative potential applied was necessary for the growth of a biofilm as the utilized microorganisms are exoelectrogenic and can immobilize on the carbon-based electrode because of their ability to take up electrons. However, the application of a constant negative potential is required for continuous CO$_2$ reduction. The reduction potential was set at $-700$ mV vs. Ag/AgCl according to the theoretical reduction potential of CO$_2$ to CH$_4$ [Eqs. (1)–(6)]. For this, the target was to use as low an overpotential as possible for the reduction to CH$_4$ and to avoid competing reduction reactions such hydrogen evolution, which occurs at even lower potentials. Biofilm formation was observed after 24 h of the application of a constant negative potential (Figure 1b). After one week, the biofilm had multiplied distinctly, and the nourishing medium was exchanged (Figure 1c).

![Figure 1](image_url)

During the biofilm formation, CH$_4$ production was monitored. Gas chromatograms during different states of the growing process are depicted in Figure 2. The increasing CH$_4$ concentration in the headspace indicates a continuous and advanced production of CH$_4$.

To characterize the biocathode, cyclic voltammetry was performed before and after the adaption was completed to investigate the redox processes associated with the microorganisms.

In the abiotic (noninoculated) MEC with a pristine carbon felt electrode, redox peaks were not detected either with N$_2$ or with CO$_2$/H$_2$ purging (Figure 3, gray line with triangles and blue line with stars). In the biotic (inoculated) nonadapted MEC a distinct increase in the reductive current from an offset of $-200$ mV vs. Ag/AgCl and a peak value at $-700$ mV vs. Ag/AgCl was observed. This reductive peak correlates with the reduction of CO$_2$ to CH$_4$ at a theoretical potential of $-0.446$ V vs. Ag/AgCl ($-0.24$ V vs. normal hydrogen electrode (NHE));
Figure 3. CVs of the biocathode in the nonadapted state. The biocathode (CF/biofilm) was characterized electrochemically with a scan rate of 1 mV s⁻¹ after purging and saturating the system with N₂ or CO₂/H₂, respectively. For comparison, the same measurements were performed for a pristine carbon felt electrode without biofilm (CF).

Figure 3, dotted red line) with a low overpotential of only 0.25 mV compared to the theoretical potential. For the N₂-purged system a small increase was observed as well (Figure 3, black line with squares), which is assumed to be because of insufficient flushing with N₂ before the measurement and the removal of CO₂ in the cathode compartment, respectively. However, the reduction current density is much higher in the case of CO₂/H₂ purging. This behavior supports the expectation of electrochemically active microorganisms established on the carbon felt electrode.

The first adaption process was performed using the technique of the successive decrease of the amount of H₂ purged through the system in three cycles. Each cycle consisted of 5 days of purging with a certain ratio of CO₂/H₂ and 2 days of no purging. During the adaption process, the CH₄ production of the microorganisms on the cathode was investigated continuously by measuring headspace gas samples by using GC, and the chromatograms of each cycle are shown in Figure 4.

Figure 4. Gas chromatograms of the development of the headspace gas constitution during adaption with CO₂ and H₂. The concentration of H₂ added was reduced with every cycle. Adaption was performed within three cycles over 3 weeks.

Even though the amount of H₂ added was reduced continuously, CH₄ generation was not affected distinctly, as can be seen from the rather uniform peak and, therefore, comparable concentration of CH₄ during all three cycles.

The long-term performance of the microbial electrosynthesis of CH₄ was investigated for the adapted MEC. The only carbon source to be reduced to CH₄ was gaseous CO₂ bubbled through the cathode compartment regularly. The CH₄ production from long-term microbial electrolysis over 22 weeks is shown in Figure 5. The CH₄ concentration in the headspace was rather constant.

In addition to CH₄, H₂ generation was observed, which is expected to be produced by the microorganisms themselves. As there was a steady potential applied to the system, we would expect water splitting—independent of the microorganisms on the electrode—with a constant generation and constant
amount of H₂. However, H₂ was not generated with a pristine carbon felt electrode at the same potential, and it is clear from the results presented in Figure 5 steady H₂ evolution was not observed. We propose that the CO₂ reduction to CH₄ using the bioelectrode occurs either through a direct electrochemical reduction because of electron uptake or through indirect electrochemical reduction because of intermediate H₂ generation by the microorganisms and subsequent conversion with CO₂ to CH₄.

Although the performance of the microbial electrolysis cell was rather constant over several weeks according to CH₄ generation from CO₂ reduction, efficiencies were low. Therefore, we tried improve the process. For this a second adaption, a different approach using glucose and an enhancement of the biofilm on the carbon felt cathode was examined.

To improve biofilm formation, more microorganism suspension was added to the cathode compartment.

For the second adaption process, glucose (0.1 mL saturated aqueous solution) was added to the cathode compartment instead of reducing the H₂ purged through the cathode compartment continuously (Figure 4). Additionally, the cathode compartment was purged regularly with CO₂. The adaption process using glucose within three cycles was monitored (Figure 6). As glucose and CO₂ served as carbon-based nutrients but the amount of glucose in the electrolyte medium was depleted during the three cycles, gaseous CO₂ was the remaining and only carbon source after adaption and, therefore, the source for the reduction to CH₄. In contrast to adaption with H₂, the CH₄ generation first increased and then stabilized at a slightly lower amount. The addition of glucose provides a simple way for adaption without the need for H₂ addition. This is favorable as H₂ can be avoided.

Cyclic voltammograms (CVs) were recorded for the characterization of the final biocathode after the completion of the second adaption with glucose (Figure 7). There is an increase in the reductive current from −300 mV vs. Ag/AgCl observed for the CO₂-saturated system (dotted red line). Saturation with N₂ and experiments without biofilm did not deliver an increase in reductive current, which indicates that the predominant reaction of the microbial electrolysis is the reduction of CO₂ to CH₄. In comparison to values obtained for CVs recorded for the nonadapted state (Figure 3), current densities are lower by approximately an order of magnitude. However, the CVs displayed in Figure 7 cannot be compared directly as not only the CV conditions were changed (only CO₂ purging instead of CO₂/H₂) but also the constitution of the mixed culture and the metabolism of the microorganisms was modified after reinculcation, second adaption, and further weeks of long-term performance. Nevertheless, the electrochemical characterization of the biocathode showed that reduction reactions that take place are only observed if CO₂ and microorganisms are both present.

The long-term performance of the final adapted biocathode was monitored by investigating the constitution of the headspace gas samples over several weeks. The gas chromatograms measured over 25 weeks after the adaption with glucose are presented in Figure 8.

As observed previously after adaption with CO₂/H₂, the CH₄ generation was rather constant during long-term performance after the glucose adaption of the biocathode. For H₂ generation, the same effects in terms of nonconstant concentrations were observed as for the first long-term investigation (Figure 5). It is expected that H₂ is not only produced by the microorganism but also partly used by the microorganism for its metabolism and the reduction of CO₂ to CH₄. The direct electrochemical reduction of CO₂ and the indirect electrochemical reduction of CO₂ using intermediate hydrogen is, therefore, not distinguishable.

The correlation of the detected amounts of H₂ and CH₄ from headspace gas samples of the cathode compartment of the MEC over that time shows that fluctuations of H₂ and CH₄ concentrations were mainly parallel (Figure 9). The peak values of concentrations for both CH₄ and H₂ were reached at approximately the same time during the long-term performance.

Potentiostatic electrolysis for 4 h at a potential of −700 mV vs. Ag/AgCl after biofilm improvement and completed adap-
tion delivered an overall Faradaic efficiency of around 22\%, calculated according to the charge consumed (39 C) and the amount of CH\(_4\) and H\(_2\) generated (17.5 µL of CH\(_4\) and 930 µL of H\(_2\)), which was detected by using GC in the headspace volume of the cathode compartment.

These results show an efficient, biological approach of CO\(_2\) reduction to a valuable fuel at rather high efficiencies and with gaseous CO\(_2\) as the only carbon source. Microbial electrocatalysis has been shown to be applicable in a long-term and continuous run with a rather constant production rate of CH\(_4\). Furthermore, the efficiency can be tuned by enhancing the biofilm formation and increasing the amount of microorganisms immobilized on the cathode. It is also expected that mixed cultures could be modified with regard to their constitution and, therefore, their ability to metabolize CO\(_2\) sufficiently to CH\(_4\) by tuning the adaption method for a CO\(_2\)-only process. These observations depict a very interesting approach for CO\(_2\) recycling. Additionally, such electrochemical processes could be driven by renewable energy sources and, therefore, represent an attractive, sustainable method for renewable energy storage.

**Conclusions**

We showed the utilization of methanogenic microorganisms, from digestate, for the reduction of CO\(_2\) to CH\(_4\) in a microbial electrocatalytic synthesis approach. Microbial electrolysis cells offer great potential for sustainable, highly efficient, and selective CO\(_2\) reduction. CO\(_2\) was reduced in a process in which microorganisms were grown on a carbon-based cathode for heterogeneous electrocatalysis. CH\(_4\) was generated by direct electron injection into biocatalysts through a direct or indirect electrochemical reduction pathway with Faradaic efficiencies of approximately 22\%. For this neither H\(_2\) carbonate, nor any other carbon source except gaseous CO\(_2\) was added artificially. In addition, no mediator or supplementary cofactors were required. This is new and different to previous studies, for example, that of Jiang et al., in which bicarbonate was used as a carbon source in the electrolyte solution.\(^{[30]}\) Therefore, they could not fully comprehend if the CH\(_4\) generation was from bicarbonate or gaseous CO\(_2\) flushed through the system or investigate the properties in a system without the addition of any carbon source. Moreover, we showed an extraordinary long-term performance of one year of continuous CH\(_4\) and H\(_2\) generation, which even extended earlier long-term investigations of, for example, van Eerten-Jansen et al.\(^{[31]}\) If H\(_2\) was produced during these electrochemical processes, it could act as a supply for the metabolism of the microorganisms. However, the direct electrochemical reduction of CO\(_2\) and the indirect electrochemical reduction of CO\(_2\) using intermediate H\(_2\) are not distinguishable.

The application of living organisms is advantageous because of their self-regeneration and adaptability to certain conditions. Here, we present a simple and efficient process that is suitable for a long-term performance of several months with continuous and stable production rates.

Additionally, an improvement of the biocathode was obtained and a second more favorable adaption technique for a CO\(_2\)-only process was investigated. Here, for the first time, two different adaption techniques were applied during a continuously running experiment.\(^{[12,30]}\) We found a convenient improvement of an existing technology that will gain interest in the topics of renewable and sustainable energy, CO\(_2\) reduction, and energy storage with biocatalysts.

**Experimental Section**

**Setup**

All experiments were performed by using a two-compartment cell with separate anode and cathode compartments to avoid the diffusion of oxygen generated anodically and the reoxidation of products generated cathodically. Both compartments were sealed with silicon septa to enable gas purging, sample withdrawal, and to guarantee anaerobic conditions for the microorganisms. Carbon felt (CF) with a size of 2.5 x 6 x 0.6 cm (the active area dipped in the electrolyte solution was approximately one third of the electrode,
and the Pt wire was not immersed in the electrolyte solution, with a Pt wire as the electrical contact served as working electrode. Pt foil was applied as the counter electrode. The potentials were applied versus a Ag/AgCl reference electrode mounted in the cathode compartment. The anode compartment contained phosphate buffer of pH 7 as the electrolyte solution and was purged with N₂ regularly to prevent oxygen diffusion. For the cathode compartment, a temperature of 30–35 °C was chosen, and a nutrient medium that consisted of phosphate buffer, vitamins, and trace elements with pH 7 was used as the electrolyte solution. The medium contained the following ingredients (per liter): KH₂PO₄ (3 g), K₂HPO₄·H₂O (2.5 g), NH₄Cl (310 mg), NaCl (130 mg), trace element solution (12.5 mL), and vitamin solution (5 mL). The trace element solution contained: HCl (25 %), 7.7 m, 10.00 mL), FeCl₃·H₂O (1.50 g), ZnCl₂ (70.00 mg), MnCl₂·4H₂O (100.00 mg), H₂BO₃ (6.00 mg), CoCl₂·6H₂O (190.00 mg), CuCl₂·2H₂O (2.00 mg), NiCl₂·6H₂O (24.00 mg), Na₂MoO₄·2H₂O (36.00 mg), and distilled water (990.00 mL). The vitamin solutions consisted of the following (per liter): biotin (2.00 mg), folic acid (2.00 mg), pyridoxine hydrochloride (10.00 mg), thiamine hydrochloride dihydrate (5.00 mg), riboflavin (5.00 mg), nicotinic acid (5.00 mg), D-calcium pantothenate (5.00 mg), Vitamin B12 (0.10 mg), p-aminobenzoic acid (5.00 mg), and lipoic acid (5.00 mg).

**Enrichment of microorganisms**

The source for the microbial inoculum was digestate collected from a wastewater treatment plant Asen (Austria). The digestate was centrifuged at 4000 rpm for 10 min. The supernatant was cultivated in headspace vials in a nutrient medium, prepared as described above, in a H₂/CO₂ atmosphere (4:1) at 37 °C under orbital shaking.

**Biofilm formation on the cathode**

For the inoculation of the microorganisms, the system was purged with N₂ gas to achieve appropriate anaerobic conditions for the methanogenic mixed culture. The enriched microorganism suspension (9 mL, 10 vol %) was inoculated into the cell. The cathode chamber was purged with CO₂ and H₂ of a ratio of approximately 1:1, and a potential of ~700 mV vs. Ag/AgCl was applied. After the clarification of the electrolyte solution caused by the attachment of the microorganisms onto the electrode as a biofilm, the medium was refreshed in the cathode compartment (Figure 1).

**Adaptation of the biocathode**

The inoculated microorganisms were first adapted by nourishing with CO₂/H₂ and reducing the H₂ amount continuously over three cycles (1:1, 2:1, and 3:1). For every adaption step, the ratio was kept for 5 days and for 5 h every day followed by 2 days of no purging. The medium of the compartment was refreshed every 2–3 weeks to provide an appropriate nutrient solution for the microorganism. Furthermore, a potential of ~700 mV vs. Ag/AgCl was applied constantly. Product generation was investigated daily by measuring the gas composition of the headspace by injecting 2 mL of headspace gas into a gas chromatograph (Thermo Scientific, Trace GC Ultra) with a gas-tight syringe. After the long-term performance of 28 weeks, microorganism suspension was inoculated into the cell to enhance the biofilm on the cathode and to improve performance of the cell. Adaptation to a CO₂-only performance (without any H₂ purging required) was then undertaken in a different approach. For this, 0.1 mL of saturated glucose solution was added, and the cathode compartment was purged with CO₂ for approximately 5 h per day. The amount of glucose in the system decreased automatically because of the microorganisms themselves as glucose was metabolized to CH₄ and, moreover, by refreshing the medium in the cathode compartment after 2 weeks to provide appropriate conditions (vitamins, trace elements) for the microorganisms.

**Electrochemical characterization**

The biofilm electrode was characterized electrochemically in its nonadapted state after the first inoculation, after the biocathode was improved, and after the second adaption with glucose was completed to a process with CO₂ as the only carbon source. Electrochemical characterization was performed by using CV. CVs were recorded after purging the cell with N₂ and CO₂/H₂ (1:1) or CO₂ respectively, for comparison. Furthermore, also a blank CF electrode was characterized by using CV under the same conditions. CVs were recorded by using a Jaisle Potentiotstat-Galvanostat IMP 88 PC-R or 1030 PCT., and electrolysis measurements were realized by using a Jaisle Potentiotstat P-M 100. For CV, potentials were swept between 0 and ~700 mV vs. Ag/AgCl with a scan rate of 1 mVs⁻¹.

**Electrolysis for efficiency determination**

Potentiostatic electrolysis at ~700 mV vs. Ag/AgCl was conducted for 24 h after the first adaption with CO₂ and H₂ and for 4 h after completed glucose adaption of the microorganisms to determine Faradaic efficiencies. CO₂ gas was the sole carbon source to be reduced sufficiently to CH₄, for both electrolysis experiments. For potentiostatic electrolysis, the cathode chamber was purged with N₂ for 1 h to ensure inert conditions and, subsequently, with CO₂ for 2 h to saturate the electrolyte solution. Headspace samples were analyzed before and after electrolysis by using GC. The charge (C) consumed during potentiostatic electrolysis was calculated from the current over time curve and correlated, by means of the eight-electron reduction of CO₂ shown in Equations (1)–(6), to the amount of CH₄ produced for the calculation of the Faradic efficiency.

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