CHEMICAL ENGINEERING TRANSACTIONS
VOL. 99, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Sauro Pierucci, Flavio Manenti

ISBN 978-88-95608-98-3; **ISSN** 2283-9216
DOI: 10.3303/CET2399052

Production of Short-chain Fatty Acid from CO_2 through Mixed and Pure Culture in a Microbial Electrosynthesis Cell

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The continuous accumulation of atmospheric CO_2 requires the development of new technologies for its mitigation. Carbon capture and utilization (CCU) technologies aim to convert CO_2 into precious compounds like chemicals and fuels. Biological fixation is an attractive CCU strategy in terms of cost, sustainability and variety of products. Chemoautotrophic microorganisms such as methanogens and acetogens are able to reduce CO_2 into acetate and methane, respectively. Acetogens bacteria are able to use CO_2 for cell growth through the Wood Liujhundal pathway, moreover, the final precursor (i.e. Acetyl-CoA) of the autotrophic metabolism, is also used in energy metabolism with acetate production as a waste product. Furthermore, it is possible to obtain multicarbon products of autotrophic origin starting from acetyl-CoA and acetate. The biotechnological use of these microorganisms requires the presence of H_2 as substrate, which is used as an electron donor in the pathway.
This reaction can be sustained by a biocathode in a microbial electrosynthesis cell, in which the reducing power is generated by a polarized electrode. This study proposes the use of a microbial electrosynthesis cell for conversion to acetate in H-cells by either a mixed culture enriched with *Acetobacterium woodii* or a pure culture of *Acetobacterium woodii*, to observe the difference in terms of acetate production and reducing power consumption efficiency. The mixed culture was obtained from a mixture of activated sludge and anaerobic digestate, treated by a protocol capable to select acetogenic microorganisms without the use of specific chemical inhibitors (2-Bromoethanesulfonate). Both inoculums were tested at room temperature (25°C) in the cathodic chamber of the H-cell at potentials in the range of -0.7 to -1.1 V vs SHE. The obtained results showed that the enriched mixed culture produced at -0.7 vs SHE a mixture of volatile fatty acids including C4 and C5 molecules with an overall coulombic efficiency of 50%, while at the potential of -0.9 vs SHE methane constituted the main product of the biocathode. The pure culture, on the other hand, showed a specific production of acetate with a coulombic efficiency of 44% at -0.9 vs SHE and 63% at -1.1 vs SHE. Furthermore, a drastic decrease in biocathode biomass was observed in pure culture, suggesting a higher tendency to form biofilms on the electrode unlike the mixed culture, which showed a standard growth profile in the bulk.

1. Introduction

One of the most important issues of these times is the climate crisis, which is generated by the continuous accumulation of atmospheric CO2 (Wyns and Beagley, 2021). From 1870 to 2015, in fact, a total of 2035 ± 205 Gt of CO2 were released into the atmosphere (Kelemen et al., 2019), resulting in an increase in global temperature of 1°C which is expected to increase by 0.2 C° every decade (Davis et al., 2018). Today it becomes more and more urgent to develop a technology capable of reducing CO2. Some technologies, defined as carbon capture use and storage (CCU) (Daneshvar et al., 2022), can convert CO2 into a product with high added value. Among them, biological processes are very attractive due to their low costs and environmental sustainability, representing an effective strategy towards a sustainable zero-emission economy. Biological reduction of CO2 through non-photosynthetic metabolisms is attractive (Gonzales et al., 2019), due to the mild conditions required.

Paper Received: 21 November 2022; Revised: 5 March 2023; Accepted: 15 April 2023
Please cite this article as: Ferretti J., Minardi R., Cristiani L., Villano M., Zeppilli M., 2023, Production of Short-chain Fatty Acid from CO_2 Through Mixed and Pure Culture in a Microbial Electrosynthesis Cell, Chemical Engineering Transactions, 99, 307-312 DOI:10.3303/CET2399052

Acetogens are organisms of great interest, as they reduce CO2 to acetate (Schuchmann and Müller, 2014; Zeppilli et al., 2016), which is very important in various industrial sectors, such as food, pharmaceutical, chemical, textile, polymer, medicine and cosmetic.This metabolic pathway is called the Wood-Ljungdahl pathway(Ragsdale, 2008), in which the reducing power is provided by molecular hydrogen. However, this electron donor is poorly soluble in the aqueous phase, therefore an alternative is the use of bioelectrochemical systems, where hydrogen is electrochemically produced directly in the biofilm formed on the biocathode (Zeppilli *et al.*, 2020). This technology currently exists only on a laboratory scale, due to the need to use chemical inhibitors (2-Bromoethanesulfonate) to inhibit competitive reactions (such as methanogenesis) (Molenaar *et al.*, 2017; Villano *et al.*, 2010). No inhibitors were used in this study, and a mixed culture from real sludge was used to promote acetogenesis, which was subjected to a series of treatments to promote and enrich spore-forming species (Diallo *et al.*, n.d.) at the expense of non-spore-forming ones. Furthermore, the mixed culture was added with pure culture of *A.woodii* by bioaugmentation approach, and were tested in parallel with pure culture of *A.woodii*. The tests were tested batch reactors at various potentials from -0.9 to -1.3 V vs SHE. However, currents are taken as reference in the paper, as the current was not always proportional to the applied potential.

2. Material and methods

2.1 Inoculum's Pre-treatment and Acetobacterium Woodii reactivation

A mixture of aerobic and anaerobic sludge from was used as starting material for the selection of autotrophic spore-forming organisms (acetogens) and to inhibit other organisms, including methanogens. The sludge was aerated for 48 hours, dried in a stove at 70°C, the dry sludge was subjected to acid shock by suspending it in an acid solution of HCl at pH 2 for 48 hours, subsequently centrifuged, the supernatant removed, and the biomass sedimented was resuspended in clostridium heterotrophic medium (GS) and reactivated at 80 °C for 10 minutes(Akhtar *et al.*, 2009). At last, *Acetobacterium woodii* 1030 (DSMZ GmbH) was inoculated as a bioaugmentation approach, resulting in a mixed culture enriched with *A.woodii*. Subsequently, the inoculum was grown on Acetobacterium medium (DSMZ GmbH) without glucose with H_2 and CO_2 as the only substrates in a 50:50 ratio to activate the autotrophic metabolism. The pH of the medium was about 7. The inoculum enriched with this procedure was carried on for about 5 months before the experiment. The pure culture was purchased from DSMZ GmbH in lyophilized form and was activated according to the protocol. The culture was maintained continuously in a 1L reactor at a temperature of 30°C in a thermostatic bath at pH 7 under heterotrophic conditions with glucose to ensure faster cell growth. A portion of medium was replaced with fresh medium for maintenance. The fresh medium was filtered (0.2 µm) and sterilized at 121°C for 20 minutes. Pure culture inoculum was collected from the glucose-reactivated culture (DSMZ procedure) which was inoculated into reactors after centrifugation and dilution with mineral medium.

2.2 Experimental set up

A microbial electrosynthesis cell (MEC) was set up in H-type cell, consisting of two 250 mL borosilicate glass bottles equipped with a side flange for the joint between the two bottles. The two bottles, namely the anodic and cathode compartments, were separated by an AEM anion exchange membrane (FUMASEP® FAS,Fumatech GmbH), which has been pretreated in a 5% w/v solution of NaCl in distilled water for 24 hours. The cell set up was operated in a configuration with three electrodes: the working electrode WE (cathode), the Ag/AgCl reference electrode and the CE counter electrode (anode). The working electrode, i.e. the cathodic chamber of the H-cell was polarized using a a IVIUM-N-STAT potentiostat in the range of -0.7 to -1.1 V vs SHE. The anode and the cathode compartments were filled up to 200 ml of the same sterilized mineral medium (see above. Finally, the gaseous phase was washed with a mixture of N_2/CO_2 (70-30% v/v).

2.3 Analytical methods and calculation

Analytical methods for CH_4, H_2, CO_2, and CH_3COO^- determination has been already described in (Cristiani et al., 2022. The acid concentration was determined by gas chromatographic analysis (Agilent GC 8860) with FID detector, by injecting a volume of 1 µL of liquid phase previously acidified with a 37% w/v phosphoric acid solution. The biomass concentration was determined through the correlation between the concentration expressed in VSSmg/L and the absorbance at 600 nm. Main calculations related to the cathodic bioelectrochemical reactions are summarized in (Ferretti *et al.*, 2022) .

3. Result and discussion

3.1 Bioelectrochemical tests at -0.7 and -0.9 V vs SHE with mixed culture

The test where the average current was about -0.5mA with a potential of -0.9V vs SHE showed the production of fatty acids up to C5 as the major product (Figure 1A). The main CO_2 reduction product resulted the acetate, which was produced from the first day of sampling and reached a maximum of 0.60 mmol/L in 5 days with a rate of 0.12 mmol/Ld. After this period the acetate concentration decreased in combination with the production of C3 and C5 carboxylic acids; indeed, propionic acid was produced starting from day 4 reaching a concentration of 0.26 mmol/L with a rate of 1.50 mmol/Ld. Isovaleric acid, which was produced from the 7th day reaching a maximum of 0.08 mmol/L with a rate of 0.04 mmol/Ld, the production of which ceased immediately afterwards. Methane, considered a byproduct, was observed starting from day 4, reaching a maximum of 0.04 mmol/Ld with a rate of 0.006 mmol/Ld. As shown in Figure 1B, the hydrogen resulted consumed only during the first 5 days in which a significative carboxylic acid production was observed. Indeed, according to the carboxylic acid consumption started at day 5, a not significative hydrogen consumption between day 5 to day 18 occurred. The biomass, as can be seen in Figure 1B, had a production rate of 0.75 mgVSS/Ld, reaching a maximum of 15 mgVSS/L measured in bulk. During the tests conducted at -1.1 V vs SHE, as shown in Figure 1C, a mixture of carboxylic acid was produced in the cathodic chamber of the test. Acetate was the main carboxylic acid produced at a rate of 0.08 mmol/Ld and reaching a maximum concentration of 0.44 mmol/L. Propionate production was observed on day 4 reaching a maximum of 0.20 mml/L with a rate of 0.06 mmol/Ld. Isovaleric, also in this test was observed on day 7°, producing a maximum of 0.07 mmol/Ld. Methane was produced from the 4th day, reaching a maximum of 8.00 mmol/L with a rate of 1.31 mmol/Ld, to then show a stationary phase and resumed production on the 14th day reaching a concentration of 10.40 mmol/L. In figure 1D it is possible to observe the trend of the biomass, which has reached a maximum concentration of 20 mgVSS/L with a rate of 1.0 mgVSS/Ld. As reported in Figure 1D the slope increase of the cumulative hydrogen was correlated to a current increase at -4.0 mA, which, in addition to generate more hydrogen, may have boosted both biomass and acetate production.

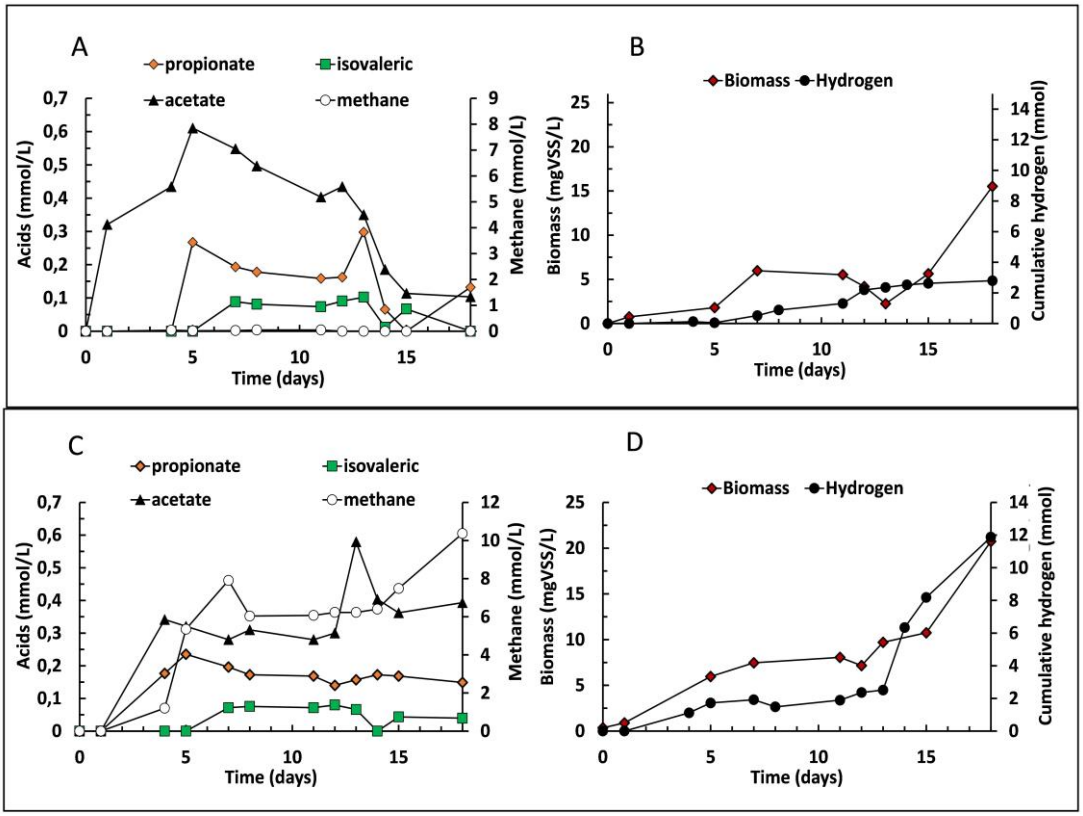

Figure 1. Carboxylic acid and methane production at -0.7 V (A) and -0.9 V (C) obtained using the enriched mixed culture. Biomass production and hydrogen consumption at -0.7 V (B) and -0.9 V (D).

3.2 Bioelectrochemical tests at -0.7 and -0.9 V vs SHE with mixed culture and *Acetobacterium woodii bioauemgentation*

The bioelectrochemical tests conducted using the *A.Woodi* pure culture were conducted with more reductive potentials corresponding to -0.9 and -1.1 V vs SHE. The use of more reductive potentials was set due to the higher internal resistance of the bioelectrochemical reactors which affected the current generation in the two bioelectrochemical reactors. Indeed, the test conducted at -0.9 V vs SHE, resulted in an average current production of - 0.05 mA, while using a potential of -1.1 V vs SHE, the resulting average current of -0.5 mA. As reported in Figure 2A, only acetate was produced during the test, moreover, an initial increase of acetate production up to 14 mmol/L (around 900 mg/L) was observed, however, considering an electron balance of the period (i.e. the coulombic efficiency for acetate production), higher than 100%, the presence of residual glucose from *A.Woodi* reactivation likely promoted mixotrophic acetate production.

Thus, for this work, rates and efficiencies are considered starting on day 5, where it was removed from the consumed medium and new medium was introduced to remove the products of fermentation from the glucose. The acetate production rate was 0.50 mmol/Ld reaching 6.5 mmol/L in days starting from 3.5 mmol/L in 6 days. The biomass production rate was 130 mgVSS/Ld, but soon after an 80% mass decrease associated with the adhesion on the electrode is observed. The hydrogen levels are very low due to the low current. (Figure 2B), also in this test only the autotrophic period is considered. However, in the test conducted at -1.1 V vs SHE, the absence of hydrogen in the headspace would suggest the presence of a mixotrophic metabolism. The autotrophic production of acetate showed a production rate of 0.55 mmol/Ld reaching the concentration of 8.70 mmol/Ld in 6 days starting from 5.00 mmol/L. Biomass production (Figure 2D), as in the test at -0.9 V vs SHE, underwent an increase with a rate of 860 mgVSS/Ld, resulting in a 90% decrease from bulk. After dilution, the production rate was 213 mgVSS/Ld, a 22% decrease was observed. Paradoxically, while the cumulative hydrogen had a low slope during the acetate production, in the last period corresponding to the biomass adhesion to the electrode, the cumulative hydrogen increased indicating biological inactivity. In both trials, the only product was acetate, confirming *A.woodii* as a selective acetate producer (Straub *et al.*, 2014). Furthermore, no methane was recorded during monitoring trials, suggesting that non-strictly sterile working conditions did not allow for contamination of the pure culture with methanogens.

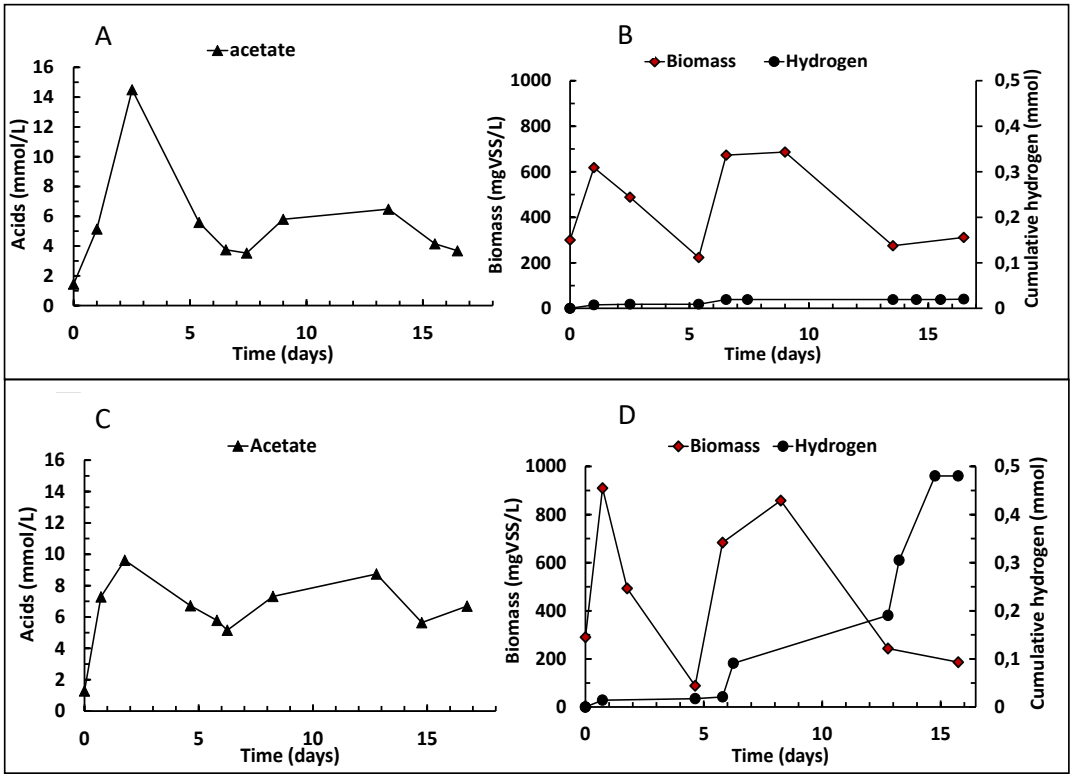

Figure 2. Carboxylic acid and methane production at -0.9 V (A) and -1.1 V (C) obtained using the Acetobacterium woodii. Biomass production and hydrogen consumption at -0.9 V (B) and -1.1 V (D).

3.3 Comparison of the bioelectrochemical tests

As resulted from the different bioelectrochemical tests, the enriched mixed culture leads to the production of different carboxylic acids starting from CO_2 and H_2 as the only substrates, while the pure culture of *A.woodii* selectively produces acetate (Straub *et al.*, 2014). The tests carried out at different potentials made it possible to discriminate the production as a function of the current. As far as the mixed culture is concerned, it is observed that working at low (-0.5 mA) current intensity it is possible to control methanogenesis, observing a total coulombic efficiency of the acids of 44.5, in which the carbon is directed more towards propionic and isovaleric, passing through acetate as a substrate for chain elongation. At high currents, on the other hand, the dominant process is methanogenesis, with an efficiency of 55.5%, against 15.5 for acids. In pure culture, on the other hand, it was possible to appreciate the effect of the current on the production of acetate, thanks to the absence of methanogens in the inoculum. In fact, it is observed that the production efficiency increases as the current intensity increases. Comparing the -0.5 mA trials, we observe that the pure culture CCE is lower than the global acid CCE of the mixed culture, generating only acetate as a CO2 reduction product. At higher current, however, the CCE of the acetate reached was 58%, while in mixed culture methanogenesis dominated.
As regards the CCE relative to the biomass, above -0.5 mA the efficiency appears to be about 20%, considering however that the mesaure was made on the biomass observed in the bulk.

4. Conclusions

In this work, the use of three different inoculum pre-treatments and augmentation for the electrosynthesis of fatty acids through the reduction of CO_2. have been reported. More in details the experimental work was conducted using a mixed culture enriched in acetogens by shock treatments, a mixed culture enriched in acetogens with bioaugmentation of A.woodii, and a pure culture of *A.woodii*., The test conducted at -0.9 V vs SHE with mixed culture showed that acid production was 150 times more predominant than methanogenesis, in fact the low current intensity of -0.5 mA proved to be a key factor in culture mixed to minimize the production of by-product methane. Furthermore, most of the electrons were engulfed in propionic and isovaleric acid. On the contrary, current intensities higher than-1.7 mA, when the potential was -1.1 V vs SHE, favored methanogenesis 3.5-fold over acid production. Pure culture, on the other hand, selectively produced acetate. Furthermore, the monitoring of the biomass in the bulk allowed us to hypothesize that the pure culture of *A.woodii* has a higher biofilm formation capacity, as a rapid decrease of the biomass in the liquid phase is observed from the first days, unlike the mixed culture showing a growth profile in the bulk. However, in all trials, an arrest of autotrophic production was found after 6 days. It could be assumed that this arrest is due to the rapid consumption of nutrients, due to the growth kinetics of microorganisms favored by bioelectrochemical systems.

Acknowledgments

Prof. Mauro Majone is acknowledged for helpful support and discussion during the experimental activity.

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