Letter

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Integrated production, extraction and concentration of acetic acid from CO_2 through microbial electrosynthesis

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Abstract

Using carbon dioxide for bioproduction combines decreased greenhouse gas emissions with decreased dependence on fossil carbon for production of multicarbon products. Microbial electrosynthesis (MES) enables this, using renewable energy to drive the reduction of CO_2 at the cathode of an electrochemical cell. To date, low product concentrations preclude cost-effective extraction during MES. Here we present an approach that couples production and recovery of acetate in a single, three-chamber reactor system. Acetate was produced at 61% coulombic efficiency and fully recovered as an acidified stream containing up to 13.5 g L^{-1} (225 mM) acetic acid, the highest obtained thus far. In contrast to previous MES studies, a single separated acidic product was generated through *in situ* membrane electrolysis enabling further upgrading.

Introduction

Carbon dioxide is ubiquitously available as a carbon source, particularly at hotspots such as steel factories. Converting this oxidized form of carbon to useful building blocks for chemical and fuel production lowers emissions of greenhouse gases to the atmosphere and decreases dependence on fossil fuel derived chemicals[1]. Microbial electrosynthesis (MES) has emerged as an attractive route that combines the advantages of electrocatalysis - electricity as source of reducing power, bringing more reducing conditions than e.g. hydrogen gas under atmospheric conditions[2] - with those of biology - the production of multicarbon products at high specificity[1]. Acetate was the dominant product of this cathodic process in the first studies with pure cultures[3, 4]. Higher concentrations (up to 175 mM, 10 g L^{-1}) were described for experiments with mixed cultures, at a somewhat lower coulombic efficiency compared to pure culture studies[5]. The use of a two-compartment setup to separate water oxidation and CO_2 reduction by a cation exchange membrane (CEM) is paradigmatic for MES studies thus far[5-7]. A major drawback of this design is that products cannot be recovered at the obtained titers, leading to possible product diversification and inhibition, while creating the need for expensive post-treatment to concentrate and acidify the products to recover them from the microbial broth. Andersen and co-workers described the extraction of carboxylates via membrane electrolysis, across an anion exchange membrane (AEM)[8]. The crossing carboxylates are protonated and recovered as volatile fatty acids (VFAs) in a clean acidic anolyte. In the present study we directly coupled the production of acetate with extraction in a single, three-chamber reactor system. This system includes an AEM separating cathode from a saline extraction compartment, and a CEM between the saline extraction compartment and the anode compartment to avoid chlorination of acetic acid at the anode. The electrical current thus simultaneously drives two processes: the reduction of CO_2 into organic carbon via homoacetogenesis and the extraction of the latter into the extraction solution (Figure 1). A fixed current was the driving force for production and extraction. Operation over several cycles shows the reproducibility of the process. The overall process design with *in situ* extraction allows stable production unhindered by product inhibition and production of acetic acid above 13 g L^{-1} (220 mM) as a single organic acid in an extraction liquid.

Materials and Methods

A three-compartment bioelectrochemical cell was constructed using Perspex frames (supplementary information, SI). All reactor compartments had a working volume of 200 mL (5 × 20 × 2 cm). An AEM (Fumatech FAB, Fumasep, Germany) separated the catholyte and extraction compartment. A CEM (Fumatech FKB, Fumasep, Germany) was used between the extraction compartment and the anolyte. Acid/base pre-treated carbon felt (thickness 3.18 mm, Alfa Aesar) with a stainless steel frame current collector was used as the cathode and Ir oxide coated titanium mesh (Magneto Special Anodes BV, The Netherlands) as anode (see SI1). All electrodes and membranes had a projected surface area of 100 cm². The initial volume of solution in each compartment was 350 mL, which includes a buffer vessel. All compartments were operated in batch mode with a recirculation rate of 31 ± 0.6 mL min^{-1}.

The catholyte consisted of a homoacetogenic growth medium with a 30 mM bicarbonate buffer at pH 7.7 (see SI2). The initial solution of the extraction compartment consisted of a 4 times concentrated salt solution containing the same salts as the catholyte, initially adjusted to pH 2 using 1 M H_2SO_4. A 50 mM Na_2SO_4 solution adjusted at pH 2 was used as anolyte to ensure sufficient initial proton migration through the CEM. A N_2/CO_2 (90%/10%) gas flow ensured anaerobic conditions in both the production and extraction compartment, CO_2 excess for autotrophic production and buffering of the catholyte. Possible volatilisation of VFAs from the extraction compartment was monitored using a 1 M NaOH trap. Antibiotics were added weekly in the anodic and extraction compartment as a precaution to avoid contamination and the associated organics consumption (see SI3).

The reactor was operated as a three-electrode setup using the cathode as working electrode. A reference electrode (Ag/AgCl, 3 M KCl, + 210 mV *vs*. SHE, BASi) was placed in the catholyte. All potentials are reported *vs*. SHE. A fixed reduction current of – 50 mA was applied using a potentiostat (VSP, BioLogic, France). A cyclic voltammogram (CV, scan rate 2 mV s^{-1}) was run before and immediately after inoculation, and once per week during experimental operation. The reactor experiments took place at room temperature (21 ± 2 °C). Control outcomes (non-inoculated with applied current and biotic without applied current) and a biological replicate are reported in SI (see SI6).

The reactor was inoculated up to a cell density of 3.4×10^6 cells $mL^{-1}_{catholyte}$ with a pre-enriched mixed microbial community. This culture was previously enriched at 28 °C from the effluent of a bioanode and anaerobic digester in serum flasks using the homoacetogenic growth medium and a H_2/CO_2 atmosphere[9]. Through serial dilution and rapid transfers in fresh medium an autotrophic acetate producing community, dominated by *Clostridiales*, was obtained which produced no methane (CH_4) even in the absence of methanogenic inhibitors.

Gas and liquid samples were taken three times per week in each compartment and an equal volume of the respective sterilized anaerobic solution was added. VFAs, alcohols and inorganic anions were measured using ion chromatography. Gas samples were measured for presence of O_2, H_2 and CH_4 using gas chromatography. Cell numbers and viability were determined once a week by flow cytometry (see SI4).

Results and discussion

In MES, carbon dioxide is reduced to VFAs as main product via the Wood-Ljungdahl pathway[3]. Production of VFAs by the pre-enriched homoacetogenic mixed microbial community started on day 3 after inoculation (Figure 2). Besides acetate we observed H_2 evolution at the cathode.

Acetate was predominantly produced in this experiment, accounting for 96.2 and 98.4 % of all VFAs present at the end of the first and second cycle, respectively (as carbon, see SI5). The remainder were low amounts of formate and propionate. From day 10 the concentration of acetate in the catholyte reached a plateau at 2.0 ± 0.5 g L^{-1} (34 ± 8 mM) while the concentration in the extraction compartment steadily rose to reach 11.9 g L^{-1} (200 mM) on day 43. The solution from the extraction compartment was changed with fresh solution to assess reproducibility of the process, starting the second cycle for extraction. The acetic acid concentration in the extraction compartment similarly increased, to 13.5 g L^{-1} (225 mM) at the end of the second cycle (Figure 2), while the concentration in the catholyte remained stable. This is the highest concentration of acetic acid reported so far for MES, and in this

case the product was already in a stream available for process use or upgrading, through esterification for example[8]. Overall 17.5 g of acetate was produced over a 86 day time period, resulting in a coulombic efficiency (CE) for production of 60.8%. When only the stable operation periods are taken into account for cycle 1 and 2 the CE was 72.6% and 67.0% respectively (Table 1). Ethanol concentrations did not exceed 35 mg L^{-1}. Electrons were lost as H_2 in the gas effluent (see further) and part of the carbon and electrons were used to sustain biomass growth, as the cell density in the catholyte increased from ~ 3.4×10^6 cells $\mathbf{mL^{-1}_{catholyte}}$ to ~ 5.5×10^9 cells $\mathbf{mL^{-1}_{catholyte}}$ after 82 days of operation. Limiting biomass growth and enhancing the catalytic effect of the microorganisms could lead to a further increase of the coulombic efficiency[1]. No organic products were detected in control experiments run in absence of either inoculum or current, showing that both the cathodic process and the enriched mixed culture are required for the system to function. An independent biological replicate furthermore had a similar production pattern, proving the technology to be reproducible (see SI6).

During the 86-day biocathode operation the pH of the catholyte remained stable at 8.4 ± 0.5. This contrasts with 2-compartment setups using a CEM where a decreasing pH is observed when VFA are produced and accumulated, an effect enhanced by migration of H^+ over the CEM[5, 7]. Using the design with AEM the pH is regulated by a balance between the different processes that either decrease (CO_2 buffering, backflux of H^+) or increase the pH (reduction of H_2O, acetate synthesis). At the start of cycle 2 the CO_2 bubbling stopped due to a technical failure. This resulted in a pH increase of the catholyte to 11.2 on day 47. The CO_2 gas flow was restored and 1 mL of 1 M HCl was dosed into the catholyte, to return the pH to under 8.5. During this intervention the production of carboxylates temporarily stopped though extraction continued, resulting in acetate concentrations as low as 194 mg L^{-1} in the catholyte on day 49. The bacterial community and acetate production rates recovered from the pH shock after 4 days.

In the abiotic tests preceding the inoculation H_2 was detected as sole product of electrochemical reduction (– 5 A m^{-2}), for a cathode potential around – 1.05 V. After inoculation a rapid consumption

of H_2 by the microorganisms took place, as H_2 was not consistently detected in the headspace gas samples over time. A cathode potential of – 1.14 ± 0.04 V *vs.* SHE was recorded during the experiment. Recovery of electrons in the form of H_2 measured in the headspace accounted for ~ 6 % of the electrons provided during the MES experiment. Microbial electrosynthesis was likely driven by indirect H_2 electron transfer, but no statements on the role of a biofilm can be made (see SI7). Biofilm formation does form an advantage when developing continuous processes, thus avoiding washout of the biocatalyst. In contrast, an active microbiome could be sustained over a long period of time in stable conditions using the present approach including product extraction. The absence of product build-up in the catholyte enabled the use of a batch mode operation for this compartment without the strict need for an electroactive biofilm and without the occurrence of product inhibition or product diversification.

The direct extraction of acetate as acetic acid constitutes the core mechanism of the technology presented here. The extraction, referred to as membrane electrolysis[8], allows a stable and continuous production by the microorganisms. The extraction efficiencies (ratio of extraction *vs.* production rates) during stable operation were greater than 94% (Table 1). The stable concentration of acetate in the catholyte is a confirmation of the efficient extraction. Acetate accounted for 8.1 ± 0.8 % of the charge passing through the AEM. Other anions present in the catholyte (mainly HCO_3^-, Cl^-, OH^-) and the backflux of H^+ through the AEM balanced the rest of the charge. This backflux is due to the non-ideal permselectivity of ion exchange membranes[10] and the pH difference between the extraction and cathode compartment (average 1.7 ± 0.2 *vs.* 8.4 ± 0.5 in the catholyte).

Acetic acid is the product of interest in this study because it can be produced at high rates via homoacetogenesis, in contrast to other carboxylates like butyrate that require chain elongation[7]. Coupling direct extraction to MES in a single, three-chamber reactor allows pure product recovery at a higher concentration than with a typical two-compartment reactor. The extracted acetic acid was obtained as a clean product in a salt solution. Operating the reactor with a smaller volume of extraction liquid would lead to a further increase of the acetic acid concentration which opens perspectives for

valorisation of the acetic acid, for example through distillation or esterification to ethyl acetate[8]. The technology presented here could contribute to a selective esterification process, in contrast to fermentation technology where a mixture of organic products is obtained[11]. The process could be sustained at a constant cell voltage of 3.6 ± 0.2 V during stable operation, indicating the robustness of the process. The specific energy input per kg of acetate produced and extracted during stable operation was 18.5 kWh kg^{-1} and 19.0 kWh kg^{-1} respectively. The energy input was calculated based on the power input and the amount of acetic acid produced or extracted for each cycle. Decreasing the anode/cathode distance below the present 4 cm distance and the use of optimized electrode materials would lead to a necessary decrease of this energy input.

Another aspect in the process optimization will be to limit acetate losses towards the anolyte. The CEM blocks negatively charged compounds but the small, uncharged acetic acid molecules were able to diffuse to the anolyte[12]. Calculated on a mass basis, 30% of the extracted acetate was present in the anolyte by the end of the second cycle (see SI5). As no oxidants other than O_2 were present and IrOx coated electrodes have high overpotentials towards organics oxidation[13], this did not result in notable losses of acetic acid. Due to the diffusive nature of this process, the concentration of acetic acid in the anode will not exceed the concentration in the extraction compartment and thus, upon prolonged operation this fraction will become limited relative to overall acetic acid production.

The operation of the reactor at – 5 A m^{-2} lead to overall acetate production rates of 0.58 g $L^{-1}_{\text{catholyte}}$ d^{-1} or 20.4 g $m^{-2}_{\text{projected cathode surface}}$ d^{-1}, and extraction rates of 19.7 g $m^{-2}_{\text{membrane surface}}$ d^{-1}. The cycles show a good reproducibility (Table 1). Comparison with other MES studies are difficult due to the high variability of MES designs and versatility of operational parameters[9]. For operation at an industrial scale the rates obtained in this study are low. Bio-ethanol production for example achieves production rates of 70 g L^{-1} d^{-1}, but this process starts from an organic substrate like corn starch[14]. Recently however acetate production rates of 148 g L^{-1} d^{-1} have been obtained in a H_2/CO_2 based fermentation with *Acetobacterium woodii*, showing that homoacetogenisis can lead to high rate bioproduction[15]. For

183 microbial electrosynthesis, optimization of the reactor design combined with increased current and the
184 use of a higher partial pressure of CO_2 as carbon source will likely increase the acetate production rate.
185
186 This is the first study presenting MES in the framework of a complete bioproduction pipeline,
187 furthermore enabling a zero-chemical-input process except for the CO_2. The possibility to
188 operate the cathode under very stable circumstances while simultaneously extracting and
189 concentrating the product as acetic acid is a key factor in the development of this technology.
190 Future research should focus on increased production rates without neglecting optimized
191 reactor design.
192
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194 The authors declare no competing financial interest
195
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204
205 ASSOCIATED CONTENT
206 Supporting Information Available: SI1. Reactor setup and current density applied, SI2.
207 Homoacetogenic growth medium, SI3. Antibiotics, SI4. Analytical techniques, SI5. Carboxylate
208 content per compartment at the end of each batch cycle, SI6. Control experiments and biological
209 replicate, SI7. Electrochemical analysis of the cathodic process. This material is available free of
210 charge via the Internet at http://pubs.acs.org.

References

1. Rabaey, K.; Rozendal, R. A., Microbial electrosynthesis - revisiting the electrical route for microbial production. *Nat. Rev. Microbiol.* **2010,** *8*, 706-716.
2. Dennis, P. G.; Harnisch, F.; Yeoh, Y. K.; Tyson, G. W.; Rabaey, K., Dynamics of cathode-associated microbial communities and metabolite profiles in a glycerol-fed bioelectrochemical system. *Appl. Environ. Microbiol.* **2013,** *79*, 4008-4014.
3. Nevin, K. P.; Woodard, T. L.; Franks, A. E.; Summers, Z. M.; Lovley, D. R., Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *MBio* **2010,** *1*, e00103-10.
4. Nevin, K. P.; Hensley, S. A.; Franks, A. E.; Summers, Z. M.; Ou, J.; Woodard, T. L.; Snoeyenbos-West, O. L.; Lovley, D. R., Electrosynthesis of Organic Compounds from Carbon Dioxide Is Catalyzed by a Diversity of Acetogenic Microorganisms. *Appl. Environ. Microbiol.* **2011,** *77*, 2882-2886.
5. Marshall, C. W.; Ross, D. E.; Fichot, E. B.; Norman, R. S.; May, H. D., Long-term Operation of Microbial Electrosynthesis Systems Improves Acetate Production by Autotrophic Microbiomes. *Environ. Sci. Technol.* **2013,** *47*, 6023-6029.
6. Jourdin, L.; Freguia, S.; Donose, B. C.; Chen, J.; Wallace, G. G.; Keller, J.; Flexer, V., A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis. *J. Mater. Chem. A* **2014,** *2*, 13093-13102.
7. Ganigué, R.; Puig, S.; Batlle-Vilanova, P.; Balaguer, M.; Colprim, J., Microbial electrosynthesis of butyrate from carbon dioxide. *Chem. Commun.* **2015,** *51*, 3235-3238.
8. Andersen, S. J.; Hennebel, T.; Gildemyn, S.; Coma, M.; Desloover, J.; Berton, J.; Tsukamoto, J.; Stevens, C.; Rabaey, K., Electrolytic Membrane Extraction Enables Production of Fine Chemicals from Biorefinery Sidestreams. *Environ. Sci. Technol.* **2014,** *48*, 7135-7142.
9. Patil, S. A.; Arends, J. B. A.; Vanwonterghem, I.; van Meerbergen, J.; Guo, K.; Tyson, G. W.; Rabaey, K., Selective Enrichment Establishes a Stable Performing Community for Microbial Electrosynthesis of Acetate from CO2. *Environ. Sci. Technol.* **2015,** *49*, 8833-8843.
10. Varcoe, J. R.; Atanassov, P.; Dekel, D. R.; Herring, A. M.; Hickner, M. A.; Kohl, P. A.; Kucernak, A. R.; Mustain, W. E.; Nijmeijer, K.; Scott, K., Anion-exchange membranes in electrochemical energy systems. *Energy Environ. Sci.* **2014,** *7*, 3135-3191.
11. Agler, M. T.; Wrenn, B. A.; Zinder, S. H.; Angenent, L. T., Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* **2011,** *29*, 70-78.
12. Vanoppen, M.; Bakelants, A. F. A. M.; Gaublomme, D.; Schoutteten, K. V. K. M.; Bussche, J. V.; Vanhaecke, L.; Verliefde, A. R. D., Properties Governing the Transport of Trace Organic Contaminants through Ion-Exchange Membranes. *Environ. Sci. Technol.* **2014,** *49*, 489-497.
13. Bagastyo, A. Y.; Radjenovic, J.; Mu, Y.; Rozendal, R. A.; Batstone, D. J.; Rabaey, K., Electrochemical oxidation of reverse osmosis concentrate on mixed metal oxide (MMO) titanium coated electrodes. *Water Res.* **2011,** *45*, 4951-4959.
14. Graves, T.; Narendranath, N. V.; Dawson, K.; Power, R., Effect of pH and lactic or acetic acid on ethanol productivity by Saccharomyces cerevisiae in corn mash. *J. Ind. Microbiol. Biotechnol.* **2006,** *33*, 469-474.
15. Kantzow, C.; Mayer, A.; Weuster-Botz, D., Continuous gas fermentation by Acetobacterium woodii in a submerged membrane reactor with full cell retention. *J. Biotechnol.* **2015,** *212*, 11-18.

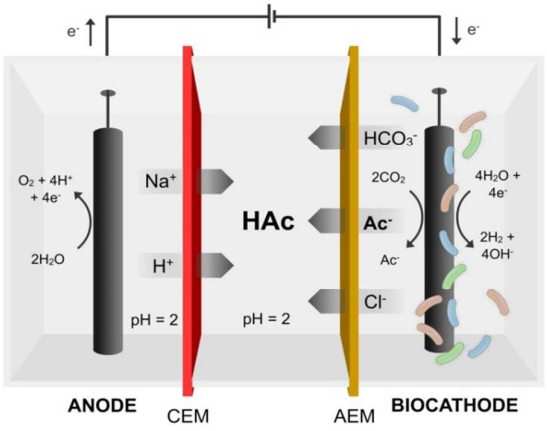

Figure 1 - Reactor concept for simultaneous biological production and extraction of acetate from CO_2 and electrical current. An anion exchange membrane (AEM) separates the cathode and extraction compartment. A cation exchange membrane (CEM) separates the anode and extraction compartment. The middle compartment serves as extraction compartment for recovery of acetate as acetic acid.

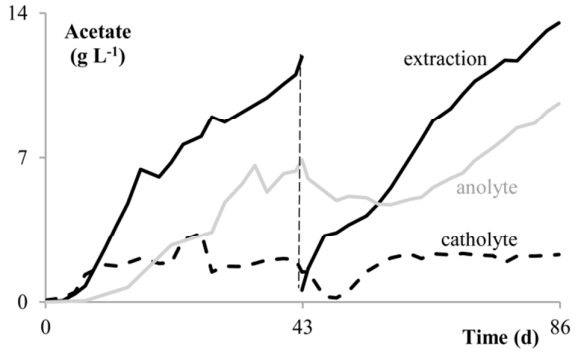

Figure 2 – The acetate concentration (g L^{-1}) in the extraction compartment steadily increased for a stable concentration in the cathode compartment. Concentrations in the catholyte (black dotted line), extraction compartment (full black line) and anolyte (grey line) are represented for a 86 day experiment. The vertical dotted line shows the start of the second cycle for the extraction compartment.

264 **Table 1 - Coulombic efficiencies, production rates (per electrode surface area) and extraction**
265 **rates (per membrane surface area) for both cycles during stable operation. The volumetric**
266 **production rates are calculated for a catholyte volume of 350 mL. The extraction efficiency is the**
267 **ratio between extraction and production rate. A mass-based graph for production is available in**
268 **SI (SI5) for interpretation of the calculated values.**

	Cycle 1 (day 10 – day 43)	Cycle 2 (day 54 – day 86)
Production rate (g L^{-1} d^{-1})	0.70	0.64
Production rate (g m^{-2} d^{-1})	24.3	22.4
CE (%)	72.6	67.0
Extraction rate (g m^{-2} d^{-1})	24.2	21.2
Extraction efficiency (%)	99.5	94.3

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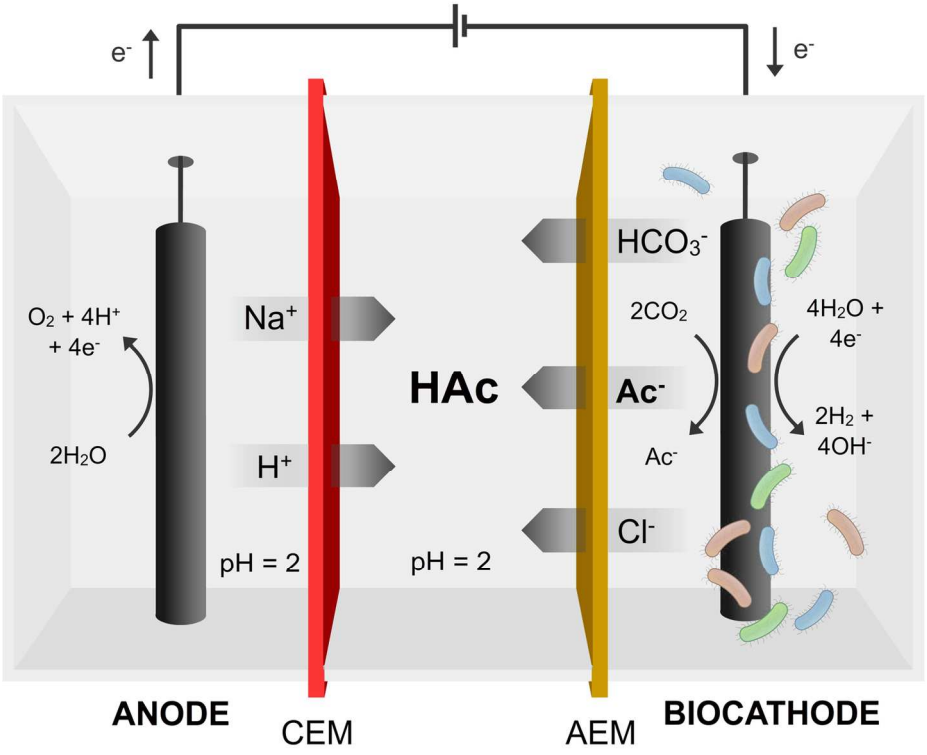

Reactor concept for simultaneous biological production and extraction of acetate from CO2 and electrical current. An anion exchange membrane (AEM) separates the cathode and extraction compartment. A cation exchange membrane (CEM) separates the anode and extraction compartment. The middle compartment serves as extraction compartment for recovery of acetate as acetic acid.
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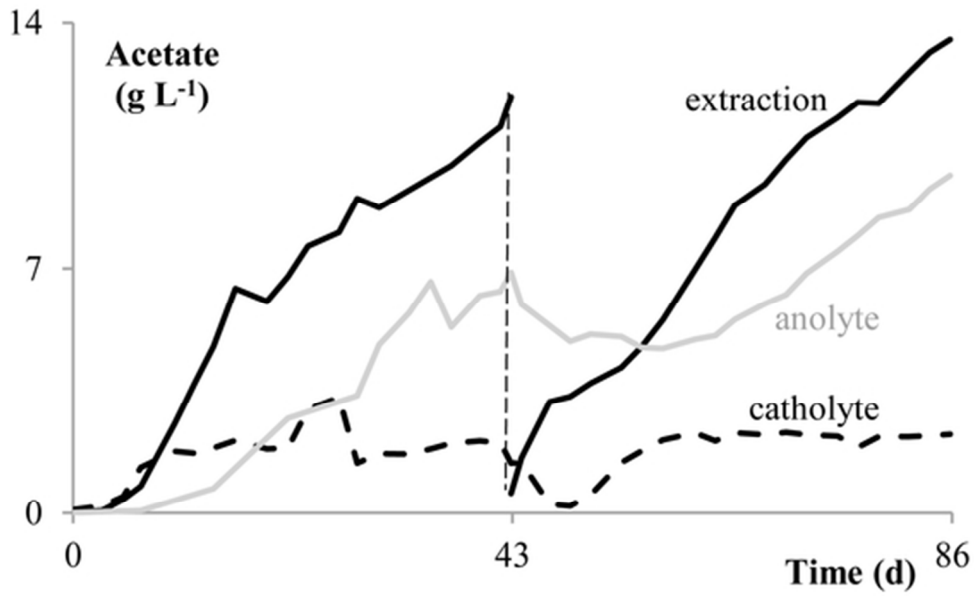
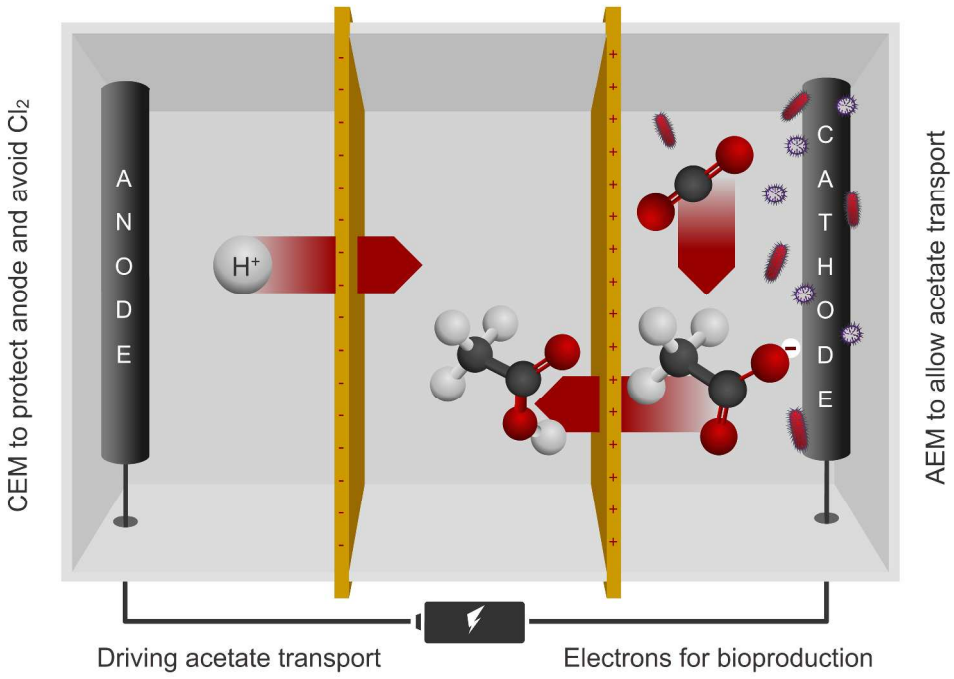


The acetate concentration (g L^{-1}) in the extraction compartment steadily increased for a stable concentration in the cathode compartment. Concentrations in the catholyte (black dotted line), extraction compartment (full black line) and anolyte (grey line) are represented for a 86 day experiment. The vertical dotted line shows the start of the second cycle for the extraction compartment.
49x29mm (300 x 300 DPI)



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