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Bioelectrochemical Production of Caproate and Caprylate from Acetate by Mixed Cultures

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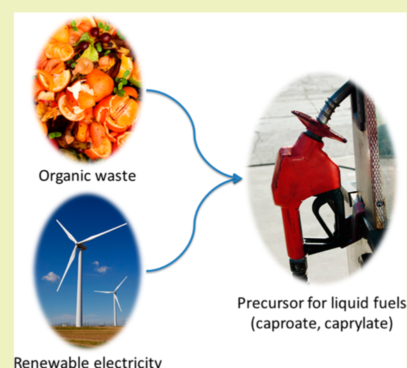
ABSTRACT: The use of mixed cultures to convert waste biomass into medium chain fatty acids, precursors for renewable fuels or chemicals, is a promising route. To convert waste biomass into medium chain fatty acids, an external electron donor in the form of hydrogen or ethanol needs to be added. This study investigated whether the cathode of a bioelectrochemical system can be used as the electron donor for the conversion of acetate into medium chain fatty acids. We show that medium chain fatty acids were produced in a bioelectrochemical system at −0.9 V vs NHE cathode potential, without addition of an external mediator. Caproate, butyrate, and smaller fractions of caprylate were the main products formed from acetate. In-situ produced hydrogen was likely involved as an electron donor for the reduction of acetate. Electron and carbon balances revealed that 45% of the electrons in electric current and acetate, and 31% of the carbon from acetate were recovered in the formed products. This study showed for the first time production of medium chain fatty acids caproate and caprylate from acetate at the cathode of bioelectrochemical systems and offers new opportunities for application of bioelectrochemical systems.



KEYWORDS: *Bioelectrochemical systems, Undefined mixed cultures, Carboxylic acids, Microbial electrolysis cell, Biocathodes, MFC, MEC, BES*

■ INTRODUCTION

Biomass is one of the main renewable sources that can be used to replace fossil-based fuels and chemicals with renewable alternatives. Biomass-derived fuels and chemicals and their production should fulfill sustainability criteria, such as decreased air pollution impact,[1] net positive energy balance,[2] no competition with food production, and lower CO_2 emissions compared to fossil-based production processes.[3] Low-grade waste biomass, such as municipal waste and crop residues, meets these sustainability criteria.[3]

Currently, anaerobic digestion is a widely used technology to convert low-grade waste biomass into renewable methane. However, attention is shifting from producing methane towards producing higher-value compounds like alcohols and fatty acids. These can, for example, be biologically produced from volatile fatty acids, key intermediates in anaerobic digestion.[4−6] Both alcohols and fatty acids are precursors for renewable fuels and chemicals. Steinbusch and co-workers found that medium chain fatty acids caproate (six carbon atoms) and caprylate (eight carbon atoms) could be produced from acetate, hydrogen, and/or ethanol by mixed cultures in (fed-)batch bioreactors.[7] Caproate and caprylate have superior physical properties for further processing to fuels and chemicals compared to volatile fatty acids or ethanol, such as a higher hydrophobicity, facilitating separation from the fermentation broth, and a lower oxygen/carbon ratio, resulting in a higher energy density.[7] Recently, it was demonstrated that caproate could be continuously extracted from the fermentation broth via liquid−liquid extraction.[8] Besides using hydrophobicity as driving force, a pH gradient was used as driving force to specifically extract fatty acids by diffusion over a membrane. For medium chain fatty acids production, however, an external electron donor in the form of hydrogen or ethanol needs to be added.

Bioelectrochemical systems (BESs) offer an opportunity for in-situ electron supply to produce all kinds of products at the cathode,[9] like hydrogen,[10] copper,[11] hydrogen peroxide,[12] alkalinity,[13] and methane.[14,15] A BES consists of two electrodes, anode and cathode, with microorganisms growing on one or both electrodes. The microorganisms on the electrode are a cheap, self-regenerating catalyst,[16] enabling all kinds of oxidation and reduction reactions that, in absence of the microorganisms, would need a more expensive catalyst to occur. In a previous study, members of our team studied

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bioelectrochemical production of ethanol from acetate.[17] Using Methyl Viologen (MV) as an electron mediator, besides the main product ethanol (83 mg/L), also butyrate (53 mg/L) was formed. Bioelectrochemical butyrate production was also recently demonstrated using Neutral Red as an electron mediator.[18] At pH 6, the maximum obtained butyrate concentration was 8.8 g/L. To the best of our knowledge, until now, there has been no report of the production of the medium chain fatty acids caproate and caprylate in BES without the use of an electron mediator.

In this study, we therefore investigated whether BESs can be used to produce medium chain fatty acids with a higher length than butyrate from acetate without the use of an electron mediator by supplying electrons or hydrogen in-situ. In this study, two electrochemical cells were compared; the cathode of the first cell was inoculated with a mixed culture, and the second cell was not inoculated and served as a control. As hydrogen possibly plays an important role to drive the production of fuels and chemicals in BESs,[9] we operated both cells at a cathode potential of −0.9 V vs NHE, which is a potential favorable for (bio)electrochemical hydrogen production.[10] This study shows for the first time that acetate can be reduced to caproate and caprylate by mixed cultures at the cathode of a BES, without the addition of an electron mediator. Acetate was mainly reduced to caproate and butyrate, and also lower concentrations of caprylate were measured.

MATERIALS AND METHODS

Electrochemical Cell. Two flat-plate electrochemical cells (0.56 L) were used as described in ref 19. The anodes were made of a platinum-coated titanium mesh (projected surface area 0.025 m^2), and the cathodes were made of graphite felt (projected surface area 0.025 m^2). The anode and cathode compartments (0.28 L each of which 0.03 L was gas headspace) were separated by a cation exchange membrane (Fumasep FKB, Fumatech GmbH, Germany). The cathode headspace was connected to a gas flow meter (Milligascounter, Ritter, Germany) via an injection port. The anode compartments of both electrochemical cells were connected via a common 10 L anolyte vessel. The electrolytes were recirculated through serpentine flow channels parallel to the electrode in both the anode and cathode compartments. The total catholyte volume (electrochemical cell, tubing, and recirculation vessel) was 0.63 L.

Inoculum and Electrolytes. One electrochemical cell was inoculated; this electrochemical cell is indicated as BES in this manuscript. The inoculum was obtained from a continuously operating anaerobic fixed film reactor operated at 30 °C in which mixed cultures, with *Clostridium kluyveri* assumed to be the predominant microorganism, produced C4−C8 fatty acids from acetate and ethanol.[20] The BES was inoculated with 5 g of reactor liquid at the start of the experiment (day 0). The biomass concentration of the inoculum was 0.2 g VSS/L at the day of inoculation.[20] As a control, the second electrochemical cell was not inoculated; this electrochemical cell is indicated as "Control".

The anolyte consisted of 100 mM potassium hexacyanoferrate(II) and 50 mM phosphate buffer and was recirculated at a rate of 10.8 L/h. The catholyte consisted of 100 mM acetic acid, 3.6 g/L $(NH_4)H_2PO_4$, 0.33 g/L $MgCl_2{\cdot}6H_2O$, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L $CaCl_2{\cdot}2H_2O$, 0.15 g/L KCl, 4 g/L K_2CO_3, 3.7 g/L NaOH, and trace metals solution and B-vitamins solution as described in ref 21 and was recirculated at a rate of 10.8 L/h. The anolyte (10 L vessel) was not replenished throughout the experiment, and the catholyte was replenished continuously at a rate of 0.16 L/d to ensure sufficient substrate. The pH of the catholyte was controlled through a pH controller (Liquisis M CPM 253, Endress + Hauser, Switzerland) at pH 6 using 2 M HCl and 2 M NaOH.

Electrochemical Cell Operation. To disinfect the reactors both electrochemical cells were flushed with 70% (v/v) ethanol, and thereafter with excess sterilized water. The catholyte of both electrochemical cells was flushed with pure nitrogen (>99.9992%) for at least 30 min. The cathodes were operated continuously during the whole experiment.

The electrochemical cells were connected to a power supply (MCP94, Bank Elektronik, Germany) to control the cell voltage. Cell voltage was adjusted to obtain a cathode potential of −0.9 V vs NHE as described in ref 22. The electrochemical cells were operated in a temperature controlled chamber at 30 °C, as this was also the temperature at which the biomass was grown.[20]

Analyses. Every 2−3 days, the composition of the catholyte and its headspace was analyzed. Gas samples were taken with a 100 μL syringe from the injection port, located between the cathode headspace and the gas flow meter. C2−C8 fatty acids and ethanol concentrations in the catholyte, and hydrogen, methane, nitrogen, oxygen, and carbon dioxide concentrations in the cathode headspace were analyzed using gas chromatography according to refs 4 and 23. Both the C2−C8 fatty acids and ethanol were measured using a gas chromatograph (HP 5890 series II GC, Germany), with a glass column packed with 10% Fluorad 431 on Supelco-port 100−120 mesh.[23] Gas production was continuously measured with a gas flow meter (Milligascounter, Ritter, Germany). The total chemical oxygen demand (COD) of the catholyte was measured using the Hach Lange LCK514 cuvette test. The principle of this test is that oxidizable substances react with sulphuric acid-potassium dichromate solution in the presence of silver sulfate as a catalyst at 148 °C for 2 h. Chloride is masked by mercury sulfate. The green coloration of Cr^{3+} was spectophotometrically analyzed at room temperature.

Calculations. We use reduced organics as the generic term for the following products that could be produced from protons or acetate: butyrate, caproate, caprylate, ethanol, hydrogen, and methane. Production of reduced organics ($p_{i,t}$ in mole e^- equiv) was calculated according to

$$\text{production}_{i,t} = \text{accumulation}_{i,t} + \text{out}_{i,t} - \text{in}_{i,t}$$

$$p_{i,t} = \left[V_{\text{cat}}(C_{i,t} - C_{i,t-1}) + Q\frac{(C_{i,t} - C_{i,t-1})}{2}\Delta t - QC_{\text{in},i}\Delta t\right]n_{e,i} \tag{1}$$

with subscript i referring to the reduced organic (that is, butyrate, caproate, caprylate, ethanol, hydrogen, or methane), V_{cat} the total catholyte volume (0.63 L), c_i the concentration of the reduced organic (mole/L), Q the influent and effluent flow rate (L/s), Δt the time difference between sample time t and previous sample time $t - 1$ (s), $c_{in,i}$ the concentration of the reduced organic in the influent (mole/L), and $n_{e,i}$ the number of electrons contained in the reduced organic (mole/mole; $n_{e,i}$ is 20 for butyrate, 32 for caproate, 44 for caprylate, 12 for ethanol, 2 for hydrogen, 8 for methane, and 8 for acetate). For each reduced organic, production was calculated according to eq 1. Total reduced organics production (in mole e^- equiv) was the sum of the production of each reduced organic. The reduced organics (both ethanol and fatty acids) that were present in the inoculated reactor liquid have been subtracted from the calculated production immediately after inoculation.

The electron equivalents for bioelectrochemical reduced organics production at the cathode can originate from two sources: electric current or acetate. Acetate can supply 8 mole of electrons per mole of acetate when it is oxidized to CO_2 and protons. The number of electron equivalents transferred via the current (q_t in mole e^- equiv) was calculated according to

$$q_t = \frac{\int_{t-1}^{t} I\,dt}{F} \tag{2}$$

with I the current (A) and F the Faraday constant (96 485 C/mole e^-).

The cathodic electron efficiency ($\eta_{ce,t}$ in percent) indicates to what extent electrons from electric current and acetate were recovered in reduced organics and was calculated according to

$$\eta_{ce,t} = \left(\frac{p_{i,t}}{q_t + p_{ac,t}} \right) 100\% \tag{3}$$

The cathodic carbon efficiency ($\eta_{cc,t}$ in percent) indicates to what extent carbon from acetate was recovered in reduced organics and was calculated according to

$$\eta_{cc,t} = \left(\frac{(p_{i,t}/n_{e,i})n_{c,i}}{(p_{ac,t}/n_{e,ac})n_{c,ac}} \right) 100\% \tag{4}$$

with $n_{c,i}$ as the number of moles of carbon per mole of reduced product (mole C/mole; $n_{c,i}$ is 2 for acetate, 4 for butyrate, 6 for caproate, 8 for caprylate, 2 for ethanol, and 1 for methane).

RESULTS AND DISCUSSION

Bioelectrochemical Production of Caproate and Caprylate from Acetate. In this study, we show for the first time that ethanol and medium chain fatty acids can be produced by mixed cultures, without the addition of an electron mediator, in the cathode of a bioelectrochemical system (BES). At −0.9 V vs NHE cathode potential, ethanol and the fatty acids butyrate, caproate, and caprylate were detected in the BES, with caproate as the predominant product (Figure 1). The concentration of reduced organics measured immediately after inoculation was corrected for the reduced organics present in the inoculum, indicating that the ethanol and fatty acids were produced as a result of microbial activity in the cathode of the BES. Besides ethanol and fatty acids, hydrogen gas was produced with hydrogen production rates ranging between 5 and 487 mL H_2/d at standard temperature and pressure (Figure 1). The control, on the other hand, did not produce ethanol nor fatty acids and produced only a small fraction of hydrogen. The corresponding current density profiles are shown in Figure 2. Current density was on average 1.8 ± 0.6 A/m^2 projected surface area for the BES, about a factor 6 higher than the average current density in the control (0.3 ± 0.2 A/m^2). The maximum production of reduced organics was found after 4 days, and production decreased thereafter (Figure 1).

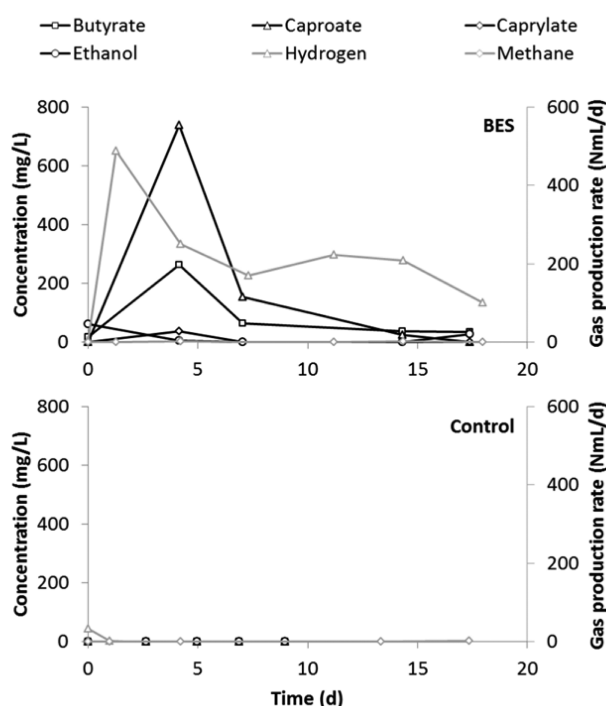


Figure 1. Concentration of reduced organics butyrate, caproate, caprylate, and ethanol, and production rates of hydrogen and methane (at standard temperature and pressure) with time at −0.9 V vs NHE cathode potential (30 °C, pH 6) for the BES and the control.

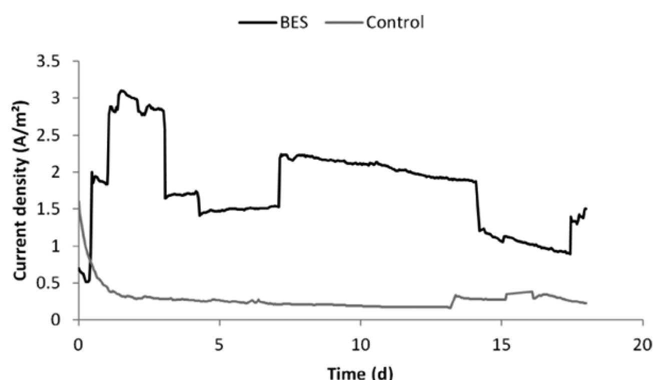


Figure 2. Current density with time for both the BES and the control.

The maximum concentrations of reduced organics were 739 mg/L for caproate, 263 mg/L for butyrate, 36 mg/L for caprylate, and 27 mg/L for ethanol. Previously reported concentrations of bioelectrochemically produced ethanol and butyrate are 83 mg/L for ethanol and 0.053−8.8 g/L for butyrate.[17,18] In the reported studies, however, an electron mediator was used to facilitate electron transfer. To the best of our knowledge, bioelectrochemical production of caproate and caprylate without the use of an electron mediator has not been reported before.

In principle, a higher current density would relate to a higher hydrogen production rate. We observed a higher current density in the BES than in the control, and indeed the hydrogen production rate in the BES was higher than in the control. The higher current density and hydrogen production rate in the BES could be a result of bioelectrochemical hydrogen production at the cathode.[10]

Cathodic Electron and Carbon Efficiency. Electron and carbon balances have been made after 18 days of operation of the BES. The electrons are available via two electron sources, the converted acetate and the electric current. These electrons were compared to the electrons in the reduced organics (ethanol, butyrate, caproate, caprylate, and hydrogen). After 18 days of operation, the average cathodic electron efficiency, the efficiency of capturing electrons from electric current and acetate in reduced organics, was 45% (Figure 3). This shows that indeed electrodes can be used for in-situ supply of electrons (or hydrogen). The largest part of the electrons was recovered in hydrogen (62%), caproate (26%), and butyrate (10%) (Figure 3). For the carbon balance, the carbon in the converted acetate was compared to the carbon in the reduced organics and was corrected for the organics in the inoculum. After 18 days of operation, the average cathodic carbon efficiency was 31% (Figure 3). The largest part of the carbon was recovered in caproate (69%) and butyrate (27%).

Here, 55% of the electrons were not recovered in the products. Possible processes leading to a loss of electrons are diffusion of hydrogen through the membrane to the anode[24] and reduction of other electron acceptors in the catholyte, such as sulfate.[25] The cathodic carbon efficiency being lower than 100% could be the result of carbon leaving the catholyte as

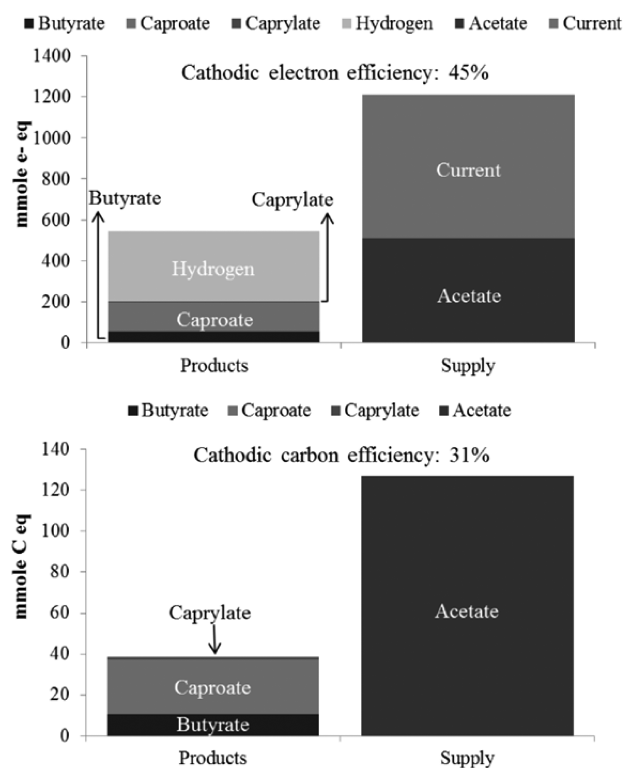


Figure 3. Electron and carbon distributions and efficiencies after 18 days of operation. The contribution of ethanol to the cathodic electron and carbon efficiencies was <1%.

another carbon-based product than the measured reduced organics. Production of other carbon-based reduced products than the ones measured (C2−C8 fatty acids and ethanol) could be ruled out as total COD tests matched the total measured carbon-based reduced products in the catholyte. Likely, the unrecovered carbon left the catholyte as CO_2 or HCO_3^-. CO_2 concentrations in the headspace did not show a clear increase in time, with an average concentration of 3.6 ± 1.2%. *Clostridium kluyveri*, assumed to be the predominant microorganism in the inoculum,[20] requires CO_2 for growth,[26] and might have consumed some of the CO_2.

Mechanisms of Reduced Organics Production. A schematic of the mechanisms of fatty acids production is depicted in Figure 4. To produce fatty acids, ethanol is required as an electron donor.[7,27] Microorganisms that produce the ethanol can use either hydrogen (pathway 2) or the cathode directly (pathway 3) as an electron donor. The hydrogen required for biological ethanol production (pathway 2) can be produced bioelectrochemically (pathway 4) or electrochemically (pathway 5). Medium chain fatty acids caproate and caprylate can be produced from butyrate (the product in pathway 1) using ethanol (the product of pathways 2 or 3) as an electron donor, as described in more detail in ref 27. This study cannot decide on the dominant mechanism.

Most likely, the dominant mechanism of fatty acids production was via hydrogen (pathways 4 and 5). At −0.9 V vs NHE cathode potential, both electrochemical hydrogen production and bioelectrochemical hydrogen production via hydrogen-producing microorganisms that can use the cathode as electron donor can take place.[10,28]

Direct electron transfer from the cathode to microorganisms for fatty acids production has until now only been

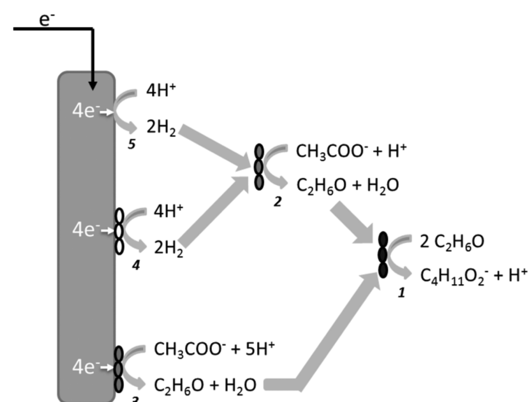
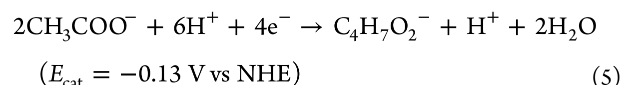


Figure 4. Schematic of the mechanisms of fatty acids production. Ethanol is required as electron donor for fatty acids production (pathway 1). Microorganisms that produce the ethanol can use either hydrogen (pathway 2) or the cathode directly as electron donor (pathway 3). The hydrogen required for biological ethanol production from acetate (pathway 2) can be obtained from bioelectrochemical hydrogen production (pathway 4) or electrochemical hydrogen production (pathway 5).

demonstrated once.[29] *Sporomusa ovata* was able to directly accept electrons from an electrode potentiostatically controlled at −0.4 V vs NHE to convert CO_2 to acetate and small amounts of 2-oxobutyrate.[29] Direct electron transfer from the cathode to microorganisms for longer chain fatty acids production has not been demonstrated. Fatty acids production using the cathode directly as electron donor would offer a considerable advantage compared to production via hydrogen, as the required energy input would theoretically be lower, for instance 0.23 V lower for butyrate production compared to (bio)electrochemical hydrogen production (E_{cat} = −0.36 V vs NHE at 30 °C and pH 6) (eq 5).



$$2CH_3COO^- + 6H^+ + 4e^- \rightarrow C_4H_7O_2^- + H^+ + 2H_2O \quad (E_{cat} = -0.13 \text{ V vs NHE}) \qquad (5)$$

The thermodynamic cathode potential was calculated, using the Gibb's free energies as described in ref 30, and using 30 °C, pH 6, and 1 M for acetate and butyrate.

Further investigation of the mechanisms and studying the possibility for steering toward a direct mechanism, would therefore be an interesting topic for further study.

Perspectives. This study showed for the first time that caproate and caprylate could be produced at the cathode of a BES. Compared to fermentation processes that use externally added electron donors, supplying electrons in-situ in a BES has several advantages: (i) no infrastructure for supply of hydrogen is required, (ii) in-situ electron supply at the right cathode potential might result in locally high hydrogen partial pressures, which are favorable for the reaction, and (iii) the electron donor is renewable when renewable electricity is used.

Important elements to be addressed in future studies are (i) continuous production of medium chain fatty acids, (ii) choice of a suitable electron donor at the anode, and (iii) separation of the medium chain fatty acids from the catholyte.

Electron transfer for the bioelectrochemical production of reduced organics can occur via two mechanisms: via direct extracellular electron transfer or via electron mediators produced by the microorganisms.[31] The production of reduced organics stagnated after 18 days of operation, possibly caused

by biomass or electron mediator wash-out due to the continuous flow conditions. Therefore, ways to retain the biomass, such as increasing the solid retention time, or a (fed-)batch system should be further investigated. Alternatively, continuous addition of inoculum could prolong reduced organics production. This principle has already been applied in continuous *n*-butanol fermentation, where the biomass cells were grown in a separate continuous stirred tank reactor and continuously added to the fermentation broth.[32] This resulted in an extension of continuous *n*-butanol production from 7 days to over 2 months.[32]

Hexacyanoferrate(II) was used as an electron donor at the anode. Hexacyanoferrate(II) was chosen to prevent the possible crossover of acetate (in the case of a bioanode) or oxygen (in case of water oxidation) from anode to cathode. Further work needs to include sustainable alternatives for hexacyanoferrate(II) such as water or organic matter. Using water has the advantage that it is a widely available and cheap resource, however, a higher electrical energy input is needed compared to using domestic wastewater as electron donor,[33] and diffusion of the produced oxygen to the cathode[34,35] could negatively affect organics production. Using domestic wastewater has the advantage that a lower electrical energy input is needed compared to using water as electron donor; however, it will lead to a loss in overall carbon efficiency because part of the acetate is oxidized to CO_2. The feedstock price and the price of electricity will determine what will be the most attractive electron donor in the future.

For implementation of the technology, it is important that a renewable source of acetate is used for the production of medium chain fatty acids at the cathode. Acetate is a key intermediate in anaerobic digestion. In anaerobic digestion, acetate is finally converted into methane. Production of medium chain fatty acids instead of methane from acetate has, however, higher energetic and economic value than producing methane from acetate.[7] Sustainable sources that could be anaerobically digested to acetate are low grade wet biomass sources, such as the organic fraction of municipal solid waste.[7]

In this study, a mixture of reduced organics was produced, with caproate as the main component. To increase the applicability and economic value of the produced products, concentration and purification is required. Separation steps, however, require an additional energy input. Besides, the almost similar physical properties (e.g., charge, hydrophobicity) of C2−C6 fatty acids poses a challenge for selective separation of reduced organics from the fermentation broth. To decrease the energy input for separation, it would be interesting to study if further concentration and selective production of specific reduced organics is feasible. Variability in the feedstock composition, and the use of mixed cultures to deal with this variable feedstock composition, puts challenges on selective production of specific reduced organics.[36] Increasing the production rate, and the concentration of the reduced organics, is probably the key challenge for implementing the technology.

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Notes

The authors declare no competing financial interest.

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