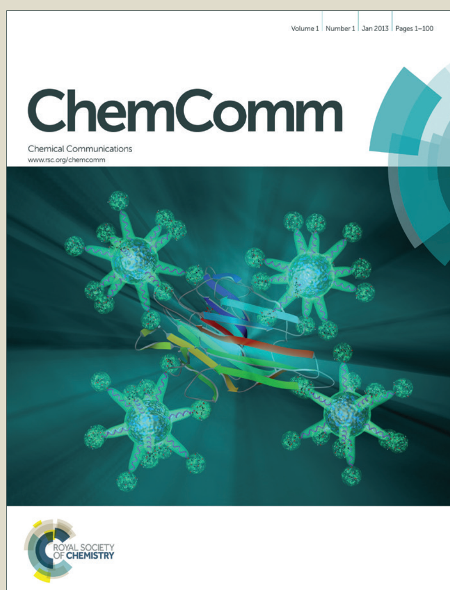
# ChemComm

Accepted Manuscript




This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm

RSCPublishing

COMMUNICATION

Cite this: DOI: 10.1039/x0xx00000x

# In-line and selective phase separation of medium-chain carboxylic acids using membrane electrolysis

Jiajie Xu,[a] Juan J.L. Guzman,[a] Stephen J. Andersen,[b] Korneel Rabaey[b] and Largus T. Angenent*[a]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

ChemComm Accepted Manuscript

**We had extracted *n*-caproate from bioreactor broth. Here, we introduced in-line membrane electrolysis that utilized a pH gradient between two chambers to transfer the product into undissociated *n*-caproic acid without chemical addition. Due to this acid's low maximum solubility, selective phase separation occurred, allowing simple product separation into an oily liquid containing ~90% *n*-caproic and *n*-caprylic acid.**

The United States produced ~50 billion L of ethanol from corn kernels in 2013; an additional ~60 billion L of ethanol or ethanol-equivalent fuel has been mandated by 2020 from more recalcitrant cellulosic feedstocks.[1, 2] Distillation, which is the primary method for extracting the completely miscible ethanol product from fermentation beer, is one of the major energy consuming steps, resulting in a considerable energy tax on the usable energy in the ethanol product.[3] In addition, many U.S. ethanol plants use molecular sieves to separate the ethanol from the remaining water due to the azeotrope. The energy tax shows that even for existing bioprocessing platforms, product extraction and separation remains problematic. As an alternative to ethanol distillation, we had converted left-over biomass, yeast cells, and dilute ethanol in fermentation beer into the medium-chain carboxylate *n*-caproate, which we extracted from the bioreactor broth with a considerably lower energy tax than ethanol distillation.[4] However, the resulting dissociated *n*-caproate product (R-COO$^-$) is not in a very useful form and further conversion to the undissociated form (R-COOH) with conventional processes would require additional chemicals to the hydroxide that was already added for the in-line extraction. Here, our objective was to test a novel approach to separate medium-chain carboxylic acids (MCCAs) without the addition of chemicals such as salts.

MCCAs are saturated fatty acids, which are 6 to 12 carbons long (*n*-caproic acid = *n*-hexanoic acid [C6]; *n*-enanthic acid [C7]; *n*-caprylic acid [C8]; etc.), and feature relatively low maximum solubilities in their undissociated form, allowing recovery by phase separation after acidification. MCCAs are currently harvested from plant oils and animal fats and are used to manufacture products, including fragrances, pharmaceuticals, food additives, antimicrobials, lubricants, rubbers, and dyes.[5, 6] Recent research has also proposed to convert MCCAs into liquid biofuels through esterification and hydrogenation.[7] Besides the many uses and the longer chain length, the advantage of MCCA oil compared to distilled ethanol is the more than double price.

In our work, we employ microbiomes (open cultures of microbial consortia) in bioreactors to produce MCCAs through reverse beta-oxidation by elongating short-chain carboxylic acids (SCCAs; C2-C5) with ethanol as a source of reducing equivalents, energy, and a chain of two carbon atoms.[8-11] Moreover, we utilize complex organic-rich byproducts as feed substrates such as fermentation beer from the corn kernel-to-ethanol industry.[4, 12, 13] We have also used real syngas fermentation effluent to elongate SCCAs.[14, 15]

In our work with fermentation beer conversion, we had utilized an in-line membrane liquid-liquid extraction (*i.e.*, pertraction) system to selectively extract MCCAs from our bioreactor broth (pH=5.5) into an alkaline (pH=9) extraction solution (Supplementary Information).[4] Pertraction is a low energy extraction process, primarily requiring energy to pump the bioreactor broth, mineral oil solvent, and alkaline extraction solution. We achieved a dissociated *n*-caproate concentration of 58 g $L^{-1}$, which is higher than the maximum solubility of the undissociated *n*-caproic acid in water (10 g $L^{-1}$). However, at the alkaline conditions in the extraction solution, no phase separation occurred, because with a pKa of 4.88 the product is primarily in the dissociated *n*-caproate form. The extraction solution must be maintained alkaline within this extraction system to maintain a pH gradient (5.5-9.0) and an undissociated MCCA concentration gradient, which is the driving force for diffusional transport over the membranes through the selective mineral oil solvent. Therefore, for every undissociated MCCA molecule that crosses into the extraction solution, an $OH^-$ molecule had to be added to maintain alkaline conditions *via* pH control.

This journal is © The Royal Society of Chemistry 2012

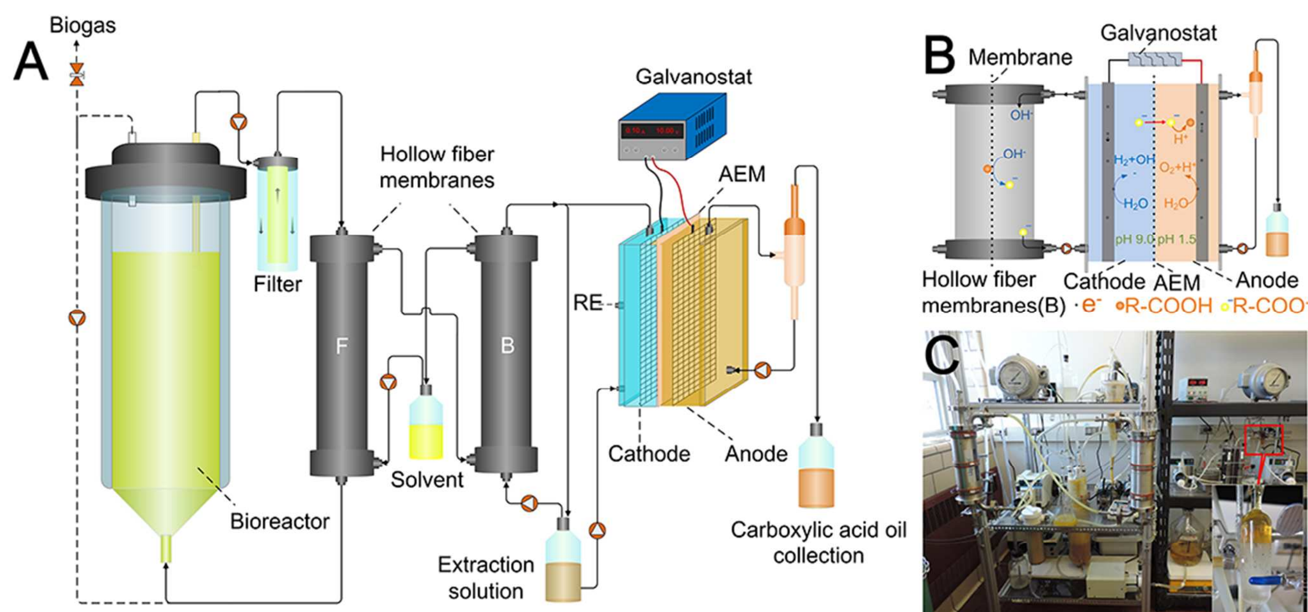



Fig. 1 Schematic of the bioreactor and product separation system. A: schematic of the bioreactor, membrane liquid-liquid extraction (pertraction) system, and membrane electrolysis cell. Key notes: solid lines, liquid streams; dashed lines, gas streams; AEM, anion exchange membrane; RE, reference electrode; F, forward extraction module; and B, backward extraction module. B: detailed mechanism of anion transfer and collection in the membrane electrolysis cell. C: picture of actual bioreactor and product separation system.

To harvest a useful product from the extraction solution, however, the pH must be lowered to achieve phase separation of the undissociated MCCA. Here, we integrated a membrane electrolysis cell, which performed *in-situ* phase separation and pH control, with the published MCCA bioreactor and pertraction system,[4] to continuously separate MCCAs as an oil without the need to input chemicals for extraction and separation (Fig. 1A). Our 5-L MCCA-producing bioreactor with pertraction has been continuously operated for over three years, resulting in a microbiome shaped for MCCA production. This bioreactor was being fed with ethanol fermentation beer in a semi fed-batch mode (15 day HRT), while controlling the pH at 5.5 and a temperature of 30°C (Fig. 1A; Table S1). The bioreactor broth was recirculated through the forward extraction module of the pertraction system for 45 min out of every h.[4] Simultaneously, the alkaline extraction solution from the backward extraction module of the pertraction system was continuously recirculated through the cathode of the membrane electrolysis cell, making the alkaline extraction solution and catholyte the same solution (Fig. 1; Supplementary Information).

The membrane electrolysis cell is an abiotic electrochemical cell. Previously, electrodialysis cells have been used to concentrate SCCAs, such as acetic, lactic, and *n*-butyric acid, from aqueous solutions.[16-20] In addition, a membrane electrolysis cell has been tested to extract acetate from fermentation broth.[21] However, these cells have not been used to concentrate or separate MCCAs nor have they accomplished phase separation without chemical addition. Here, we present a membrane electrolysis cell that consisted of cathode and anode compartments (wet cathode volume: 270 mL; wet anode volume: 240 mL) separated by an anion exchange membrane (AMI-7001S, Membranes International Inc., Ringwood, NJ) (Fig. 1B). A power supply (HY6003D, Automation Technology Inc., Hoffman Estates, IL) in galvanostatic mode (constant current) was connected to the cathode (316L stainless steel mesh; geometric dimensions: 20 cm tall × 5 cm wide; mesh width: 564 µm; wire thickness: 140 µm, Solana, Schoten, Belgium) and anode (titanium mesh electrode coated with Ir MMO; geometric dimensions: 18.8 cm tall × 4.8 cm wide × 0.1cm thick, Magneto special anodes B.V., Schiedam, The Netherlands)[22], and an Ag/AgCl reference electrode[23] was inserted in the catholyte (alkaline extraction solution). The anolyte consisted of 3 g $L^{-1}$ $Na_2SO_4$ at the start of the operating period (Supplementary Information). Before starting the electrochemical cell, the membrane was polarized for 24 h at 1/10 of the initial current density.

By applying a constant current between the cathode and anode ($e^-$ from anode to cathode), an equal flux of anions must be transferred through the anion-exchange membrane from cathode to anode to maintain charge neutrality.[22] Since the catholyte was maintained at a pH of 9 due to the electrochemical production of $OH^-$ at the cathode, the medium-chain carboxylate product was in the dissociated form and was transferred through the anion-exchange membrane into the anolyte due to charge balance forces. Once in the low pH of the anolyte (pH of ~1.5), which was maintained due to the electrochemical production of $H^+$ at the anode, carboxylates became protonated (undissociated) (Fig. 1B). The resulting MCCAs subsequently accumulated in the anolyte to reach concentrations slightly above their maximum solubility in water (*n*-caproic acid: 10.82 g $L^{-1}$; and *n*-caprylic acid: 0.68 g $L^{-1}$) at which point phase separation occurred. Then, any new medium-chain carboxylate molecule that transferred through the membrane separated from solution. Thus, the concentration of MCCAs remained slightly above the maximum solubility, while the concentration of SCCAs increased to a certain value that was lower than their maximum solubility. Therefore, we created a barrier to limit additional short-chain carboxylate molecule transfer through the membrane, ensuring a selective MCCA separation system. Anolyte was recirculated upward through the anode compartment to force phase-separated MCCA oil droplets to the top of the reactor, and to flow into an oil trap (Fig. 1). Importantly, because of the utilization of the galvanostatic power supply, the electrode potentials were high enough for electrolysis to occur, with $H_2$ and $O_2$ generation at the cathode and anode, respectively (Fig. 1B and Table S2).

We tested the membrane electrolysis cell at 5 A $m^{-2}$ during Phase 1, and at 10 A $m^{-2}$ during Phases 2 and 3. At the lower current setting (Fig. 2, Phase 1), the MCCA flux (Eq. S1) steadily increased until reaching a steady state within 18 days, achieving 244±15.7 g $m^{-2}$ $d^{-1}$

ChemComm Accepted Manuscript

 This journal is © The Royal Society of Chemistry 2012

at a voltage of 8.9±0.76 V (Days 19-30 of the operating period; Fig. 2A-B). Importantly, we were able to considerably reduce the NaOH addition for pH regulation of the extraction solution after the integration of the membrane electrolysis cells at this current (Fig. 2C). Next, we increased the current to 10 A $m^{-2}$ (Phase 2), which resulted in an MCCA flux of 488±52.5 g $m^{-2}$ $d^{-1}$ at a potential difference of 14.5±0.93 V for the first two weeks (Days 31-45; Fig. 2A-B). The automatic NaOH addition to the extraction solution/catholyte solution became zero towards the end of Phase 2 (Fig. 2C). At the end of Phase 2, we were forced to disassemble the membrane electrolysis cell because mineral oil solvent from the pertraction system clogged the cathode side of the anion-exchange membrane (Fig. S1). We installed a new membrane after which the chambers were refilled with the original catholyte and anolyte solutions (Phase 3). Our cell recovered rapidly, achieving a performance that was similar to Phase 2 within three days, with an averaged flux of 443±43.1 g $m^{-2}$ $d^{-1}$ and a voltage of 10.0±0.46 V (Days 60-83; Fig. 2A-B). Interestingly, the cell potential difference decreased immediately to similar voltages as observed during Phase 1, indicating that maintaining membrane health and minimizing undesired component transfer will increase system performance in future studies. No NaOH addition into the alkaline extraction broth occurred during Phase 3 (Fig. 1C).

We achieved an MCCA transfer efficiency, which accounts for the electric current applied to transfer MCCAs (Eq. S2-S3), of 43.5±2.52% during Days 20-30 of the operating period in Phase 1. During this time, a minimum power consumption of 4.08±0.66 kWh $kg^{-1}$ was achieved. Similar power requirements of 0.91-8.02 kWh $kg^{-1}$ have been achieved in other studies for carboxylic acid extraction, however, these were for *n*-butyric acid extraction,[24] which is a smaller molecule than MCCAs.

A maximum MCCA flux of 610±0.04 g $m^{-2}$ $d^{-1}$ and MCCA transfer efficiency of 53.9±0.34% were achieved during Days 40-42 of the operating period in Phase 2 (Fig. 2A-B). However, these did not translate to optimum electric power consumption rates due to the higher cell potential differences that were observed. During this optimum period of MCCA transfer, we measured an oil production of 6.8±0.04 mL $d^{-1}$ and a separated MCCA production rate of 6.1±0.04 g $d^{-1}$, which represented a volumetric, separated MCCA-COD production rate of ~2.5 g COD $L_{bioeactor\ volume}^{-1}$ $d^{-1}$. We believe that the MCCA flux would have been higher if the pertraction system during Phase 2 and 3 would not have limited the membrane electrolysis cell. The combined extraction/separation system reached an MCCA recovery from the bioreactor broth of ~87% during Days 40-42 of the operating period (Eq. S4). For these same days, the COD yield for the separated MCCA oil from fermentation beer influent was ~35% (Eq. S5). During the continuation of our previous study,[4] we found that a more optimum ethanol COD content in the fermentation beer increased medium-chain carboxylate COD yields significantly due to stoichiometry,[13] showing promising potential for improved performance.

We monitored the cell potential difference, cathode potential, and electric current continuously. During each of the phases, the cell potential difference gradually increased from an already high starting point, while the cathode potential remained somewhat constant at 1V (Fig. 2B). A high membrane resistance likely caused the relatively high potential differences, while the buildup of oils and solids adjacent to the membrane (*i.e.* membrane fouling) would explain the increasing potential differences that we observed (Fig. 2B). This is further demonstrated by the observation that the cell voltage immediately decreased during Phase 3 when we installed a new membrane. Again, this indicates that membrane selection will be critical for future application besides continuous membrane cleaning and maintenance.

The membrane electrolysis cell continuously separated MCCAs *via* phase separation in the anode chamber, obtaining a free-flowing oily product stream with an MCCA composition (Eq. S6) that exceeded 90% (Table S3). *n*-Caproic acid and *n*-caprylic acid dominated at 53.3±1.07% and 37.0±0.77% of the total oil composition, respectively (Days 0-24); the SCCA *n*-butyric acid accounted for only 2.62±0.21% of the total oil composition, while acetic acid concentrations were negligible. The remainder of the product (7.1±0.09%) was unidentified, but is expected to be composed of water, salts, and other metabolites. To our knowledge, this is the first study displaying the capability of a membrane electrolysis cell to continuously harvest MCCAs in a phase-separated oil with such a relatively high purity.

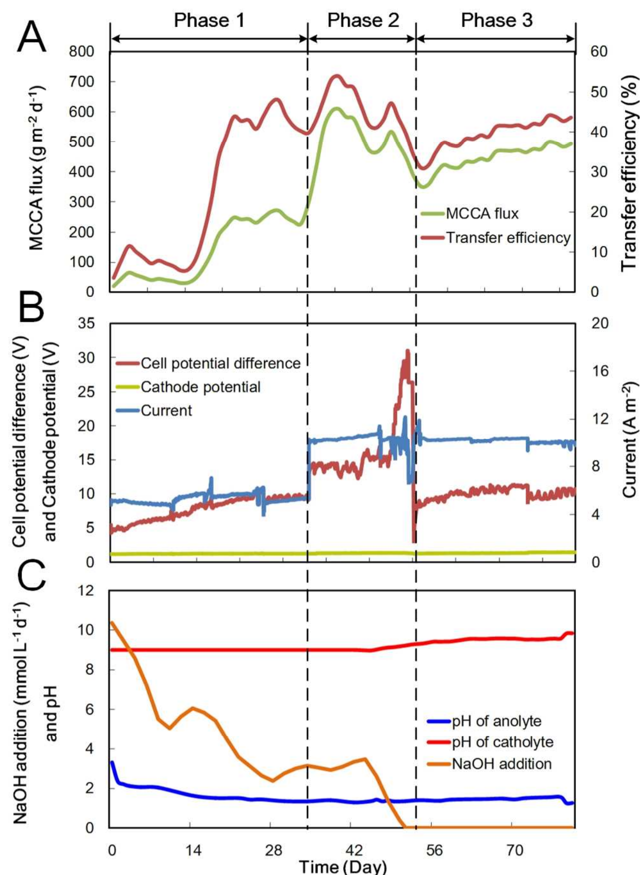



Fig. 2 Influence of parameters to the membrane electrolysis cell during three phases of the operating period. A: MCCA flux and MCCA transfer efficiency across the membrane. B: Current, cell potential difference applied, and cathode potential. C: Anolyte and catholyte pH, and NaOH addition for pH control in the extraction solution. Phase 1: current 5 A $m^{-2}$; Phase 2 and 3: current 10 A $m^{-2}$.

The extraction and separation steps (pertraction and membrane electrolysis, respectively) of our system selectively increased the proportion of the longer-chain MCCAs in the product stream. When comparing the composition of MCCAs in the bioreactor broth, pertraction effluent, and MCCA oil product, the *n*-caprylic acid percentage increased by a factor of 60 from the bioreactor broth to the extraction solution of the pertraction system, and by a factor of 2 from the extraction solution to the MCCA oil (Table S4). This was lower for *n*-caproic acid (2.0x and 0.9x, respectively), which shows that the longer chain MCCAs can be selectively extracted and separated from our bioreactor system. In other words, this selective behavior of the carboxylic acids translated to a faster flux through the two extraction and separation steps and higher relative

ChemComm Accepted Manuscript

concentrations in subsequent extraction and separation solutions for the longer-chain carboxylic acids compared to the shorter-chain carboxylic acids.

Stable microbial electrolysis cell operation led to a promising and important benefit of no longer requiring pH control by NaOH addition to maintain a pH of 9 in the extraction solution (no NaOH was added after Day 53 of the operating period; Fig. 2B). We also observed that the automatic 5 M NaOH addition to the bioreactor to maintain a pH of 5.5 was reduced from 10.5 mL $d^{-1}$ before application of membrane electrolysis (Day 0 of the operating period) to 6.25 mL $d^{-1}$ at the end of Phase 2 (Day 53 of the operating period). Thus, the *in-situ* production of $OH^-$ appears to positively affect the bioreactor operating conditions (future research would need to be performed to understand the mechanism). Therefore, the coupling of the bioreactor to the abiotic membrane electrolysis is referred to here as a secondary microbial electrochemical technology (secondary MET),[25] because the electrochemical system has a positive effect on the microbial process, reducing the automatic application of a base for pH control during acidogenesis in the bioreactor. This is critical for future applications because chemical addition of a salt (NaOH or MgOH) would be minimized, reducing costs, especially when electric power remains relatively cheap; reducing accident risks with chemical dosage; and also reducing possible problems with salt toxicity when recycling processing streams.

In the future, improving the MCCA transfer efficiency, reducing electric power requirements by reducing the cell potential differences, and obtaining stable system performance for the membrane electrolysis cell will be paramount. To improve transfer efficiency new electrochemical cell designs will be explored with optimized cell configurations. In addition, better membrane maintenance and specifically tailored membrane materials will lower its energy requirements.

## Conclusions

Here, we investigated the use of a membrane electrolysis cell that was integrated within a bioprocessing platform to continuously separate MCCAs. For the first time, we observed phase separation of a product without chemical addition by using the pH gradient between the two electrolysis chambers. The selective phase separation resulted in a continuous MCCA oil stream after bioprocessing with a starting material that was complex in nature. Importantly, only ambient temperatures and pressures are used in the carboxylate platform. The MCCAs *n*-caproic acid and *n*-caprylic acid accounted for more than 90% of the composition of the oil with a maximum oil production of 6.8 mL $d^{-1}$. The membrane electrolysis cell achieved a peak MCCA flux of 610 g $m^{-2}$ $d^{-1}$ with a MCCA transfer efficiency of 53.9% at a current of 10 A $m^{-2}$. Moreover, *in-situ* pH regulation by the membrane electrolysis cell eliminated the need to supplement the extraction (pertraction) system with base.

This work was supported by the US Army Research Office (W911NF-12-1-0555) and the US NSF (1336186) to L.T.A.. J.X. gratefully acknowledges the Chinese Scholarship Council for providing a research scholarship. J.J.L.G. acknowledges the NSF GRFP (DGE-1144153). In addition, this work was supported by ERC starter grant "ELECTROTALK" to K.R.. S.J.A. and K.R. were supported by the Multidisciplinary Research Partnership Ghent Bio-Economy. The authors wish to thank Joseph Usack and Leo Kucek for critical review, and the owners and operators of Western New York Energy (Medina, NY) for providing corn beer.

## Notes and references

[a] Department of Biological and Environmental Engineering, Cornell University, 226 Riley–Robb Hall, Ithaca, NY 14853, USA.

[b] Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium.

Electronic Supplementary Information (ESI) available: Supplementary materials and methods, Equations S1-S5, Tables S1-S4, and Figure S1. See DOI: 10.1039/c000000x/

1 Renewable Fuels Association, *Annual Industry Outlook*, 2014.
2 U.S. Congress, *Title II, Section*, 2007, **201**.
3 L. Bessa, M. C. Ferreira, E. A. C. Batista and A. J. A. Meirelles, *Energy*, 2013, **63**, 1-9.
4 M. T. Agler, C. M. Spirito, J. G. Usack, J. J. Werner and L. T. Angenent, *Energy Environ Sci*, 2012, **5**, 8189-8192.
5 E. Sauer, *Kirk-Othmer Encycl Chem Technol*, 1992.
6 B. S. Jeon, C. Moon, B. C. Kim, H. Kim, Y. Um and B. I. Sang, *Enzyme Microb Technol*, 2013, **53**, 143-151.
7 F. Goembira, K. Matsuura and S. Saka, *Fuel*, 2012, **97**, 373-378.
8 C. M. Spirito, H. Richter, K. Rabaey, A. J. M. Stams and L. T. Angenent, *Curr Opin Biotech*, 2014, **27**, 115-122.
9 K. J. Steinbusch, H. V. Hamelers, C. M. Plugge and C. J. Buisman, *Energy Environ Sci*, 2011, **4**, 216-224.
10 T. Grootscholten, K. Steinbusch, H. Hamelers and C. Buisman, *Bioresour Technol*, 2013, **135**, 440-445.
11 M. T. Agler, B. A. Wrenn, S. H. Zinder and L. T. Angenent, *Trends Biotechnol*, 2011, **29**, 70-78.
12 M. Agler, C. Spirito, J. Usack, J. Werner and L. Angenent, *Water Sci Technol*, 2014, **69**.
13 S. Ge, J. G. Usack, C. M. Spirito and L. T. Angenent, *Environ Sci Technol*, Submitted.
14 D. Vasudevan, H. Richter and L. T. Angenent, *Bioresour Technol*, 2014, **151**, 378-382.
15 H. Richter, S. E. Loftus and L. T. Angenent, *Environ Technol*, 2013, **34**, 1983-1994.
16 X. L. Wang, Y. M. Wang, X. Zhang, H. Y. Feng and T. W. Xu, *Bioresour Technol*, 2013, **147**, 442-448.
17 A. M. Lopez and J. A. Hestekin, *Sep Purif Technol*, 2013, **116**, 162-169.
18 A. Vertova, G. Aricci, S. Rondinini, R. Miglio, L. Carnelli and P. D'Olimpio, *J Appl Electrochem*, 2009, **39**, 2051-2059.
19 J. J. Du, N. Lorenz, R. R. Beitle and J. A. Hestekin, *Sep Sci Technol*, 2012, **47**, 43-51.
20 P. Pal, J. Sikder, S. Roy and L. Giorno, *Chem Eng Process*, 2009, **48**, 1549-1559.
21 S. J. Andersen, T. Hennebel, S. Gildemyn, M. Coma, J. Desloover, J. Berton, J. Tsukamoto, C. Stevens and K. Rabaey, *Environ Sci Technol*, 2014, **48**, 7135-7142.
22 J. Desloover, A. Abate Woldeyohannis, W. Verstraete, N. Boon and K. Rabaey, *Environ Sci Technol*, 2012, **46**, 12209-12216.
23 D. P. Webster, M. A. TerAvest, D. F. R. Doud, A. Chakravorty, E. C. Holmes, C. M. Radens, S. Sureka, J. A. Gralnick and L. T. Angenent, *Biosens Bioelectron*, 2014, **62**, 320-324.
24 Y. Wang, X. Wang, R. Tang, S. Yu, B. Zheng and Y. Feng, *J Mol Catal B: Enzym*, 2010, **66**, 294-301.
25 U. Schröder, F. Harnisch and L. T. Angenent, *Energy Environ Sci*, 2015.

ChemComm Accepted Manuscript


This journal is © The Royal Society of Chemistry 2012