1st International Chain Elongation Conference

Connecting Communities

Book of Abstracts

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International Chain Elongation Conference 2020

Book of Abstracts

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Website

Date Monday 26 October 2020 until Tuesday 27 October 2020

Venue Virtual Conference hosted from WUR campus

Contact Associate Professor David Strik
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Local organising committee

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Dr. Tom Sleutels, Wetsus - european centre of excellence for sustainable water technology, The Netherlands

Prof. dr. Dong Hongmin Institute of Environmental and Sustainable Development in Agriculture (IEDA) & Chinese Academy of Agricultural Sciences (CAAS), China

A. Prof. dr. Sebastià Puig University of Girona (Spain)

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Thanks for participating

On Monday and Tuesday, the 26th and 27th of October 2020 we had the pleasure to organize the first international Chain Elongation Conference. Despite most of us being stuck at home due to lockdown measures, we were pleased with the degree of togetherness that we all were able to summon in the digital space of the world wide web. We witnessed 50 inspiring presentations, of which there were 6 done by invited speakers. In between presentations we had interactive discussions and many digital meetups. In total there were 208 attendees that used the brella.io platform to interact during the conference.

We started this endeavor with the intention to help connect the communities around the world that are working on chain elongation. We are glad that scientists, pioneering and established industries, governmental organizations, and other stakeholders had the chance to get more connected to the world of chain elongation by participating in this conference. Let us all continue to contribute to the inspiring research field of microbial chain elongation and facilitate the transition towards a more sustainable world.

Within the book most of the presenter abstracts are displayed, including DOI numbers that can be used to refer to the works. Aside from this book, there is also a digital depository of the presentations, which can be found at https://library.wur.nl/ojs/index.php/ICEC2020/index.

Scope

Chain-elongation bioprocesses are part of the carboxylate or waste-biorefinery platforms. Chain elongation is emerging as a pertinent bioprocess within the Circular Economy to recover resources. Microbial chain-elongation processes allow the conversion of numerous (in)organics feedstocks (from C1 compounds onwards) into a variety of fatty acids or carboxylic acids, alcohols, alkanes, and other biochemicals.

Currently, organic waste streams are digested into methane, which is typically converted into electric power, or even just flared off. Chain elongation does allow utilization of such waste streams into more valuable products. Various products are formed with diverse properties, which allow numerous applications (e.g., animal feed, antimicrobials, biofuels, platform chemicals). Pioneering research work and the current expansion of chain elongation research is leading towards mature applications.

Fundamental research, system analysis, separation technology development, as well as bioprocess engineering is ongoing. Several companies performed pilot tests and are making great progress to scale-up specific chain elongation bioprocesses. We are at an excellent moment in time to bring together the research community to share and connect with the goal to further develop chain elongation into an industrially relevant biotechnology production platform.

Themes

All topics related to chain elongation are welcome and are categorized in the following 3 domains:

Biorefinery development & integration in Circular Economy

to include CO_2 capture, pre-treatment, separation, purification, conversion of chain elongation products, application, LCA, TEA, system analysis, or pilot-studies and beyond.

Bioreactor engineering and bioprocess development

to show news on feedstock use, products, selection pressure, operating conditions, bioreactors, modelling, etc.

Microbial physiology, pathways, informatics, genetics

to gain insight about microbiomes and pure- and co-cultures and additional in-depth analysis of the biological fundamentals such as microbial physiology.

Invited speakers

Niels van Stralen, Msc.

(key-note)
Director and co-founder of ChainCraft B.V.

Amsterdam, the Netherlands

Biosystems Engineer. Co-founded ChainCraft as spin-off company from the Enviromental Technology group of Wageningen University. Currently ***ChainCraft*** *is operating a fully equipped demo-scale factory in the Port of Amsterdam, The Netherlands.*

Prof. Dr. Diana Machado de Sousa

(key-note)
Microbiology

Wageningen University, the Netherlands

Biological Engineer and microbiologist. As lead of the Microbial Physoplogy Group she is searching for ***new anaerobic microorganisms and their networks****. She e.g. constructed new co-cultures on several chain elongation processes.*

Prof. Dr. Mark Holtzapple

(key-note)
Department of Chemical Engineering

Texas A&M University, USA

Chemical Engineer. Developed a wide variety of technologies on chemicals, food and water production. One of his inventions is the ***MixAlco Process*** *which converts biodegradable wastes into products like alcohol fuels. These findings are under commercial deployment by* Eart Energy Renewables*.*

Prof. Dr. ir. Lars T. Angenent

(invited)
Max Planck Institute for Developmental Biology

University of Tübingen, Germany

Environmental biotechnologist. One of his interests is the recovery of carbon with open cultures (reactor microbiomes), defined mixed cultures, or pure cultures of microbes. He is promoting the ***carboxylate platform*** *as an important platform in biorefineries because water and nutrients must be recycled while bioenergy yields must be maximized. He leading one of the pioneering groups on Chain Elongation and is entrepreneur as e.g. scientific advisor and co-founder of* Capro-X*.*

Prof. Dr. Ramon Ganigué

(invited)
Center for Microbial Ecology and Technology

Ghent University, Belgium

Environmental biotechnologist. Interested in anaerobic microbial processes, such as C1 fermentations, chain elongation, microbial electrosynthesis, etc. In his research he aims to understand the physiology of microbes and their interactions within a microbiome as cornerstone to the development of new bio-processes.

Associate Prof. Dr. Piotr Oleskowicz-Popiel

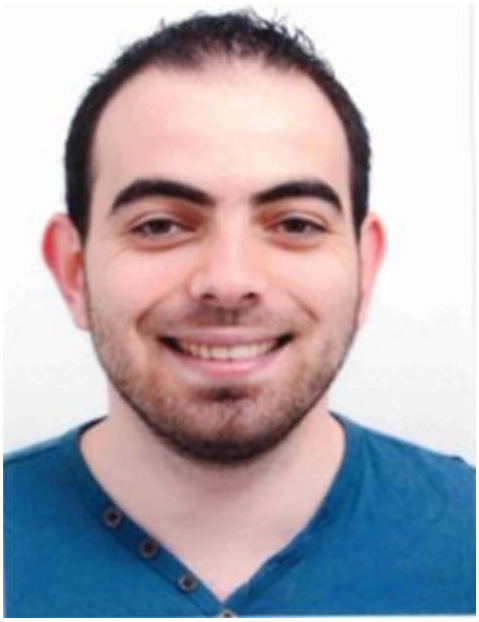
(invited)

Water Supply and Bioeconomy Division

Poznan University of Technology, Poland

Chemical engineer. Focused on biorefinery approach to convert waste streams into valuable products. He is using open culture fermentation ***to recover carbon*** *in a usable and attractive form. He developed a caproic acid production process which he aims to commercialize in the near future.*

Dr. Ludovic Jourdin



(invited)

Department of Biotechnology

Delft University of Technology, the Netherlands

Environmental biotechnologist. Focuses on developing microbial electrochemical technologies to convert waste streams into valuable products. His main interest has been microbial electrosynthesis of chemicals from C1 compounds to date, e.g. to produce hexanoate. His group applies a multiscale approach investigating phenomena from nanometre to metre scales, and from fundamental to applied research.

Prof. Dr. Byoung-In Sang

(invited)

Department of Chemical Engineering

Hanyang University, Korea

*Chemical Engineer. Interested in anaerobic fermentation and -omics study of **chain elongation**, microbial electrosynthesis, power to methane, and biodegradable plastics. He developed medium-chain carboxylic acids production process integrated with the catalytic conversion process for n-alkanes, alcohols, ketones, and ester compounds from MCCAs.*

Prof. Daniel R. Noguera

(invited)

Department of Civil and Environmental Engineering

Wisconsin Energy Institute, University of Wisconsin, Madison

Environmental Engineer. His research focuses on engineering microorganisms and developing microbiome approaches to valorize residues form the lignocellulosic biorefinery industry, such as lignin and stillage. Interested in genome-scale modeling to elucidate the microbe-microbe interactions occurring in chain elongation microbiomes. Also works on energy-efficient biological nutrient removal from wastewater.

Program

26th October Monday

Opening	Associate Prof. David Strik (Chairman)	09:00 AM
Key-notes		
Key-note presentation: Presentation Chaincraft	Niels van Stralen (Keynote speaker)	09:15 AM
Key-note presentation: Medium chain carboxylic acids production by chain-elongation (CE) process	Prof. Wanqian Guo (Keynote speaker)	09:45 AM

Biorefinery development & integration in Circular Economy

CAPRA: piloting biological chain elongation of syngas-based bio-ethanol	Fabian de Wilde	10:15 AM
Networking break		10:30 AM

Biorefinery development & integration in Circular Economy II

Invited presentation: The lactate platform	Prof. Largus Angenent (Invited speaker)	10:45 AM
Production of caproic acid from fruit waste at pilot scale	Fernando Silva (Speaker)	11:00 AM
Direct medium-chain carboxylic-acid oil separation from a bioreactor by an electrodialysis/phase separation cell	Dr. Jiajie Xu (Speaker)	11:15 AM
Role of dynamic membrane development on chain elongation for medium chain carboxylic acids production	Shilva Shrestha (Speaker)	11:30 AM
Highly selective recovery of medium chain carboxylates via anion exchange chromatography and CO2-expanded methanol desorption	Clara Fernando Foncillas (Speaker)	11:45 AM
Networking break		12:00 PM

Bioreactor engineering and bioprocess development I

Invited presentation: Resource recovery from organic waste – a short story about bioprocess development	Associate Prof. Piotr Oleskowicz-Popiel (Invited speaker)	01:00 PM
Optimum caproic acid production initiated from homogenised and acidified granular sludge is influenced by lactic:butyric acid ratio and concentration	Corine Nzeteu (Speaker)	01:15 PM
Characterization of carboxylate producing microbial communities	Dr. Nathaniel Fortney (Speaker)	01:30 PM
Mildly acidic pH selects for chain elongation over propionic acid production in lactic acid fermentation	Dr. Pieter Candry (Speaker)	01:45 PM
H2/CO2 gas recirculation to improve lactate-to-caproate selectivity	Flávio Baleeiro (Speaker)	02:00 PM
Flow cytometry as tool to monitor chain elongation performance	Kevin Sabbe (Speaker)	02:15 PM
6x scale-up while maintaining stable production of n-caprylic acid	Dr. Byoung Seung Jeon (Speaker)	02:30 PM
Networking break		02:45 PM

Bioreactor engineering and bioprocess development II

Invited presentation: Medium chain fatty acid (MCFA) production in a lignocellulosic biorefinery	Prof. Daniel Noguera (Invited speaker)	03:00 PM
Continuous carbon chain elongation from one carbon compounds	Patrick Schweizer (Speaker)	03:15 PM
Machine learning-assisted identification of bioindicators predicts medium-chain carboxylate	Bin Liu (Speaker)	03:30 PM

production performance of an anaerobic mixed culture		
Identifying chain elongation processes during the mixed-culture fermentation of proteins	Riccardo Bevilacqua (Speaker)	03:45 PM
Enriching Microbiomes for the Production of Branched Medium Chain Carboxylates and Alcohols	Kasper de Leeuw (Speaker)	04:00 PM
Retrofitting agricultural biogas plants into biorefineries – medium-chain carboxylates production from crop silage	Dr. Heike Straeuber (Speaker)	04:15 PM
Networking break		04:30 PM

Bioreactor engineering and bioprocess development POSTERS I

Poster presentation: Medium-chain carboxylic acids production using consortia from winery wastewater, ruminal fluid and granular sludge	Sharon Villegas-Rodríguez (Speaker)	04:45 PM
Poster presentation: Effect of domestication and microbial community on medium chain fatty acids production from Chinese liquor distillers' grain	Yujia Lin (Speaker)	04:50 PM
Key-note		
Key-note presentation: Carboxylate platform; Conversion of biomass to chemicals and fuels	Prof. Mark Holtzapple (Keynote speaker)	05:00 PM
QUIZ		05:30 PM
Networking break		06:00 PM

27th October Tuesday

Key-note

Key-note presentation: Production of carboxylates by microbial co-cultures growing on syngas	Prof. Diana Sousa (Keynote speaker)	09:00 AM

Microbiology, pathways, informatics, genetics I

Revamping the model CO-fermenting acetogen, Clostridium autoethanogenum, as a CO2-valorisation platform	James Heffernan (Speaker)	09:30 AM
Genomic and metabolic features of three novel Clostridia isolates involved in lactate-based chain elongation	Dr. Sabine Kleinsteuber (Speaker)	09:45 AM
The isolate Caproiciproducens sp. 7D4C2 produces n-caproate at mildly acidic conditions from hexoses: genome and rBOX comparison with related strains and chain-elongating bacteria	Dr. Sofia Esquivel-Elizondo (Speaker)	10:00 AM
Methanol-based chain elongation of acetate to i/n- butyrate at thermodynamic equilibrium of isomerization by an enriched microbiome	Kasper de Leeuw (Speaker)	10:15 AM
Networking break		10:30 AM

Microbiology, pathways, informatics, genetics II

Invited presentation: Multi-omics study on chain elongation processes for medium chain carboxylic acids	Prof. Byoung-In Sang (Speaker)	10:45 AM
Production of isobutyric acid from methanol by Clostridium Luticellarii	Camille Petrognani (Speaker)	11:00 AM
The occurrence and ecology of microbial chain elongation of carboxylates in soils	Prof. Anca Delgado (Speaker)	11:15 AM
Simulating chain elongation with constraint-based metabolic modelling	Assistant Prof. Matthew Scarborough (Speaker)	11:30 AM

Microbiology, pathways, informatics, genetics POSTERS

Poster presentation: Co-culture of Lactobacillus and Megasphaera species to produce caproic acid by mimetic microbiome system using food wastes	Hyunjin Kim (Speaker)	11:45 AM
Poster presentation: Caproic acid production from lactate using Megasphaera hexanoica	Seongcheol Kang (Speaker)	11:50 AM
Networking break		12:00 PM

Bioreactor engineering and bioprocess development III

Invited presentation: Chain elongation – friends and foes	Prof. Ramon Ganigue (Invited speaker)	01:00 PM
Understanding oscillation in gas fermentation	Dr. Esteban Marcellin (Speaker)	01:15 PM
Microbial recycling of biodegradable plastic PLA (poly lactic acid) into a spectrum of bioplastic precursors by anaerobic fermentation	Associate Prof. David Strik (Speaker)	01:30 PM
The effect of nano zero-valent iron on chain elongation	Xindi Fu (Speaker)	01:45 PM
Impact of substrate concentration on granular fermentation for caproic acid production	Quinten Mariën (Speaker)	02:00 PM
Food waste served three ways: butyric, lactic or caproic acid	Vicky De Groof (Speaker)	02:15 PM
Effects of zero-valent iron nanoparticles on lactate-based chain elongation	Carlos Contreras Davila (Speaker)	02:30 PM
Networking break		02:45 PM

Bioreactor engineering and bioprocess development IV

Invited presentation: Microbial electrosynthesis - a techno-economic driven roadmap towards implementation	Assistant Prof. Ludovic Jourdin (Invited speaker)	03:00 PM

Boosting a biocathode by analysis: the invasive effects of cyclic voltammetry on bioelectrochemical chain elongation	Sanne de Smit (Speaker)	03:15 PM
Bio-electroCO2recycling to C4-C6 products in two steps	Meritxell Romans (Speaker)	03:30 PM
The role of inorganic carbon in a hydrogen-based Membrane Biofilm Reactor	Diana Calvo (Speaker)	03:45 PM
Harnessing Hydrogen Production during Microbial Chain Elongation for Reduction of Oxidized Groundwater Contaminants	Aide Robles (Speaker)	04:00 PM
Modelling fermentative hydrogen production of cheese wastewater	Thiago Ravanini do Nascimento (Speaker)	04:15 PM
Networking break		04:30 PM

Bioreactor engineering and bioprocess development POSTERS II

Poster presentation: Parameters affecting chain elongation from syngas bioconversion	Carla Fernández (Speaker)	04:45 PM
Poster presentation: Medium Chain Fatty Acids Production Integrated with Continuous Biohydrogen Process: A Closed Loop Approach	Dr. Naresh Amradi (Speaker)	04:50 PM
Round Table - Connecting Communities	Associate Prof. David Strik (Moderator) Niels van Stralen (Panelist) Dr. Juan Guzman (Panelist)	05:00 PM
Closure	Associate Prof. David Strik (Chairman) Prof. Cees Buisman (Panelist) Prof. Largus Angenent (Panelist)	05:30 PM

	Prof. Ramon Ganigue (Panelist)	
Networking final - see you next time!		06:00 PM

Presentations

Key-note presentation: Presentation Chaincraft

Niels van Stralen - Director and co-founder of ChainCraft B.V.

DOI: https://doi.org/10.18174/icec2020.18000

Key-note presentation: Medium chain carboxylic acids production by chain-elongation (CE) process

Prof. Wanqian Guo - Dean of Department of Environmental Engineering, Harbin Institute of Technology - School of Environment State Key Laboratory of Urban Water Resource & Environment - China

Biorefinery development & integration in Circular Economy

CAPRA: piloting biological chain elongation of syngas-based bio-ethanol

DOI: https://doi.org/10.18174/icec2020.17999

Authors

Mr. Fabian De Wilde - OWS nv

Dr. Sylvia Gildemyn - OWS nv

Mr. Jan Smis - OWS nv

Abstract

HIGHLIGHTS:

- Syngas-based bio-ethanol production is coupled to biological caproic acid production to obtain high added-value products from CO_2.
- After studying operational parameters at 5L-scale, the process was upscaled to a 125L bioreactor
- Continuous in-line extraction of the caproic acid allows recovery of caproic acid bio-oil for demonstration of down-stream applications

BACKGROUND: Carbon Capture and Utilisation (CCU) technologies now deliver a wide range of products with varying amounts of incorporated CO_2. The downstream processing of these products after an initial production step can be challenging. Initial CCU products are furthermore typically limited to C1 and C2 hydrocarbons, for example ethanol in case of a biological reduction process. To tackle both these issues simultaneously, a biological chain elongation technology can transform the short chain products of an initial syngas fermentation step into caproic acid bio-oil, a longer chain product that readily phase-separates. This avoids an energy-intensive distillation step for product recovery. The coupling of syngas fermentation and biological chain elongation has been demonstrated at lab-scale[1]. To bring forward this biological chain elongation technology, several technical challenges remain, including the definition of

optimal operational conditions and scale-up challenges. The CAPRA project brings together industry partners and research institutes – ArcelorMittal, OWS, Proviron, Ghent University and VITO – to develop a biological chain elongation process at pilot scale, including: 1) in-line product recovery; 2) development of downstream processing routes to fine chemicals; and 3) an extensive life-cycle assessment and techno-economic assessment of the process.

The specific objectives of the work performed at OWS were: 1) the definition of the optimal substrate ratio (ethanol/acetic acid ratio) for the mixed culture bioprocess; and 2) the scale-up of the bioprocess for the production of minimum 50 kg caproic acid bio-oil, at a production rate of 10 g/L/d.

RESULTS & DISCUSSION: A lab-scale bioreactor, inoculated with an enriched mixed microbial culture, was operated continuously using substrate with a varying molar ratio of ethanol/acetic acid: 3, 6, 10, 20. At steady state conditions, and for three independent time periods, the molar ratio of 3 resulted in the highest carbon conversion efficiencies, here calculated as the sum of butyric, caproic and caprylic acid measured in the reactor broth (Figure 1). This increase in conversion efficiency was mainly a consequence of increased butyric acid production at lower ratios. These findings confirm previous reports, stating that lower molar ratios favour higher conversion rates, while higher molar ratios favour longer chain products[1, 2]. This observation is related to the increased ATP production at higher ethanol/acetic acid ratio's, which increases the feasibility of the chain elongation reaction, while the lower ethanol/acetic ratio probably decreases biomass production, favouring the product output[1, 3].

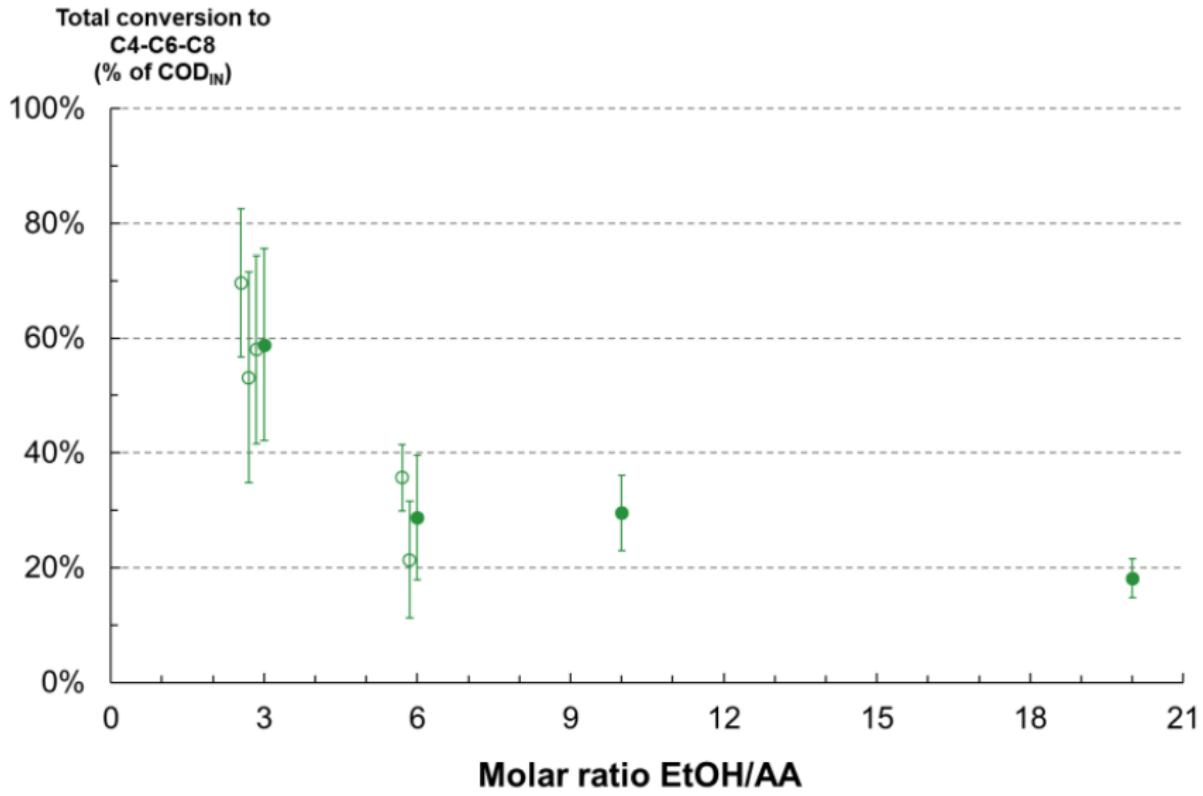


Figure 1. The total conversion efficiency of ethanol and acetic acid to butyric, caproic and caprylic acid was highest at a molar ratio of 3, with a decreasing trend at higher ethanol/acetic acid ratios.

Based on the conclusions from lab-scale research performed by OWS and Ghent University, a pilot-scale reactor was designed to scale up the bioproduction process. This pilot includes continuous product recovery via in-line membrane-based pertraction. The continuous removal of caproic acid can positively affect the process, by avoiding product inhibition and by increasing the driving force for the production of the more hydrophobic longer chain products.

The pilot scale bioreactor consists of a 125L biological compartment, a biomass filtration system to obtain cell-free permeate, and a pertraction system in which the permeate contacts the extraction solvent, following the principles of a system described previously for a lab-scale reactor[1]. The bioreactor was operated continuously using the optimal operational parameters defined earlier in the project. The initial three months of operation resulted in the production of almost 20 kg caproic acid. A loading rate increase will result in a production rate of 10 g/L/d and the production of at least 50 to 100 kg extracted caproic acid bio-oil by December 2020. This production phase deepens the general understanding of biological chain elongation under realistic operational

conditions. The extracted caproic acid bio-oil will allow the demonstration of downstream processes, will greatly improve the marketability of the chain elongation process, and will create a non-palm-based caproic acid market.

CONCLUSION: This study represents a major step forward in bringing biological chain elongation closer to the market. The combination of lab-scale research on operational parameters and pilot activities under realistic conditions are a unique asset. CAPRA contributes to the advancement of CCU technologies and to solving challenges related to chain elongation technology.

Invited presentation: The lactate platform

Prof. Largus Angenent - Max Planck Institute for Developmental Biology University of Tübingen, Germany

DOI: https://doi.org/10.18174/icec2020.18003

Production of caproic acid from fruit waste at pilot scale

Authors

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Ms. Daniela Pequito - Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

Mr. Nuno Marques - Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

Prof. Gilda Carvalho - Advanced water management centre, The University of Queensland

Prof. Maria Reis - Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

Abstract

HIGHLIGHTS:

- Apple fruit waste was used as a feedstock for a fermentation process
- A caproate-rich fermentate was obtained with an acidification yield of 56%
- Caproate productivity of 8.94 gCOD L^{-1} d^{-1} was obtained

BACKGROUND: 86 million metric tonnes of apples were produced worldwide in 2018, out of each about 23 % in Europe. Also, according to FAO, about 27% of those don't even make it to the supermarket which leaves over 5 million metric tonnes of waste in Europe alone to be treated[4]. Since apple fruit waste is mainly organic, biodegradable and with high chemical oxygen demand (COD), an

approach to treat this waste could consist on anaerobically fermenting it to produce added-value products such as hydrogen and medium chain fatty acids (MCFA) in the scope of a sustainable biorefinery. While hydrogen can be use essentially as fuel and reagent for the Haber-Bosch process, caproic acid is currently used as a platform chemical to manufacture products such as fragrances, pharmaceutical compounds, food additives among others. Moreover, it has been proposed that caproate could be converted into a liquid biofuel[3, 4]. In this frame, a 100 L upflow anaerobic sludge blanket (UASB) reactor was inoculated with granules from a full-scale anaerobic digestor with the aim of fermenting apple fruit waste into caproate by means of chain elongation reactions. DNA sequence analysis was carried out to identify the microbial profile of the caproate-producing community.

RESULTS & DISCUSSION: As represented in Figure 2, the UASB was operated during 154 days with a fixed hydraulic retention time of 1 d and a variable organic loading rate: started at 5 gCOD L^{-1} d^{-1} in day 0 and gradually increased up to 27.9 gCOD L^{-1} d^{-1}. This slow increase enabled the gradual acclimatization of the anaerobic mixed culture to the operating conditions. Butyrate and valerate were the predominant volatile fatty acids produced in the first 46 days of acclimatisation. The following phase consisted of a transition phase when the reactor effluent shifted to a fermentate rich in caproic acid. The end of this transition phase marks the end of a residual production of methane and sudden increase in hydrogen concentration in the gas outlet stream (data not shown).

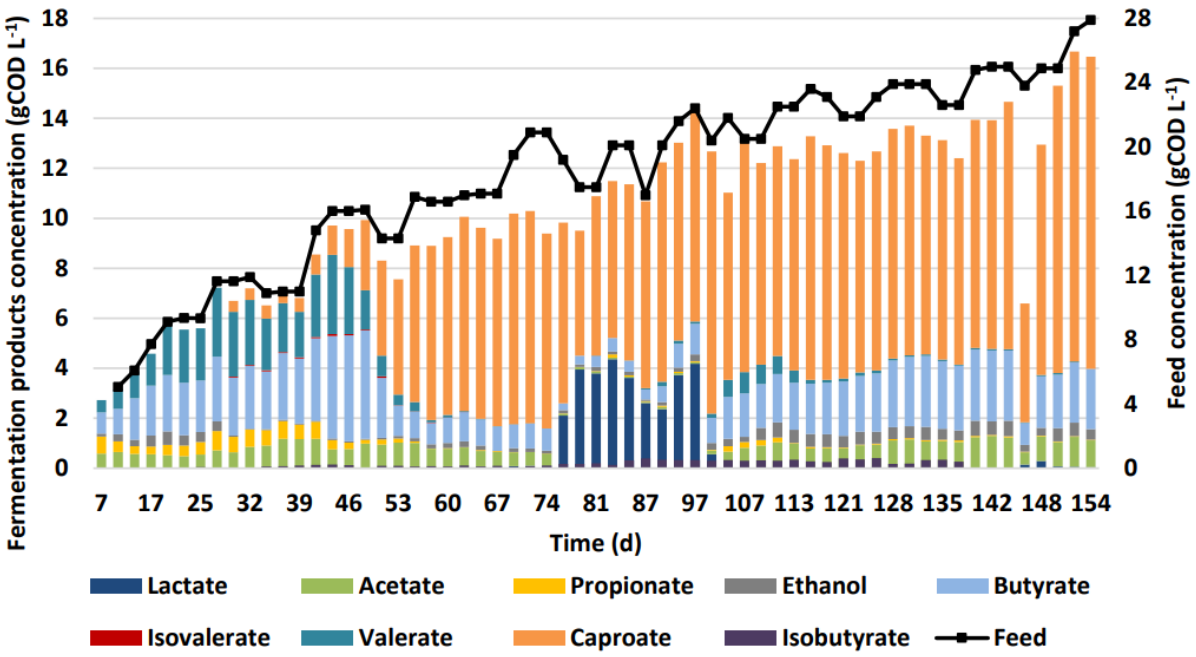

Figure 2. Trend of the concentration of the fruit waste and fermentation products (FP) in the reactor outlet.

From this point on, the effluent composition changed again. Elevated hydrogen partial pressure has been reported as an inhibitor of competing processes for MCFA formation such as their oxidation as well as ethanol oxidation[5]. Up until the end of operation, the UASB produced a fermentate consisting of caproate (68.9 ± 4.14%), butyrate (17.3 ± 3.44%), acetate (7.13 ± 1.78%), ethanol (3.63 ± 0.77%), valerate (2.04 ± 2.34%), propionate (0.63 ± 0.62%) and lactate (0.40 ± 0.99%), on a COD basis. Caproate productivity of 8.94 ± 0.69 gCOD L^{-1} d^{-1} as well as a yield of 39.1 ± 3.01 % were achieved. The gas outlet in this phase had a flow rate of 0.43 L min^{-1} and contained hydrogen (33%) and carbon dioxide (67%). DNA sequence analysis showed the microbial cultures present in the bulk as well as the one in the granules were dominated by the genera *Ruminiclostridium 5* and *Atopobium*. These genera have previously been associated with chain-elongation microbial processes[6].

CONCLUSION: The present work demonstrates a way of valorising apple waste into hydrogen and caproic acid. According to recent literature, this study stands among the studies reporting the highest productivities for caproate production so far, and more specifically, the highest caproate productivity reported using mixed microbial cultures and fermented waste in single-stage processes with no extraction of caproate from the broth[7]. Additionally, a considerable amount of

hydrogen is produced as by-product opening a window for the development of a sustainable biorefinery process.

ACKNOWLEDGMENTS: The authors are thankful for the financial support from Fundação para a Ciência e Tecnologia (Portugal) for funding through PD/BD/126626/2016 and PD/BD/104767/2014 and UCIBIO financed by national funds from FCT/MCTES (UIDB/04378/2020).

Direct medium-chain carboxylic-acid oil separation from a bioreactor by an electrodialysis/phase separation cell

DOI: https://doi.org/10.18174/icec2020.18005

Authors

Dr. jiajie Xu - Cornell university

Dr. Juan Guzman - Cornell university

Prof. Largus Angenent - Center for Applied Geosciences, University of Tübingen

Abstract

we tested an ED/PS cell, which, when evaluated in series with pertraction, achieved a maximum MCCA-oil flux of 1,665 g d^{-1} per projected area (m^2) (19.3 mL oil d^{-1}) and a MCCA-oil transfer efficiency [100%*moles MCCA-oil moles $electrons^{-1}$] of 74% at 15 A m^{-2}. This extraction system demonstrated a ~10 times lower electric-power consumption of 1.05 kWh kg^{-1} MCCA oil when compared to membrane electrolysis in series with pertration (11.1 kWh kg^{-1}

MCCA oil) at 15 A m^{-2}. Second, we evaluated our ED/PS as a stand-alone unit when integrated with the anaerobic bioprocess (without pertraction), and demonstrated, for the first time, that we can selectively extract and separate MCCA oil directly from chain-elongating bioreactor broth with just an abiotic electrochemical cell. We assumed that such a stand-alone unit would reduce capital and operating costs, but electric-power consumption increase considerably due to the lower MCCA concentrations in the bioreactor broth compared to the pertraction broth. Only a full techno-economic analysis will be able to determine whether the use of the ED/PS cell should be as a stand-alone unit or after pertraction.

Role of dynamic membrane development on chain elongation for medium chain carboxylic acids production

Authors

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Ms. Siqi Xue - University of Michigan

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Abstract

HIGHLIGHTS:

- An anaerobic dynamic membrane bioreactor produced MCCAs from food and brewery waste and resulted in effective solids liquid separation
- A suspended solids removal efficiency of ³ 95% was achieved without membrane cleaning or replacement
- The formation of a dynamic membrane enhanced MCCAs production

BACKGROUND: Chain elongation is an emerging anaerobic biotechnology that uses mixed microbial communities for organic waste stream conversion into medium-chain carboxylic acids (MCCAs). MCCAs are platform chemicals with diverse industrial and agricultural applications[3]. A product recovery system is needed to recover MCCAs in a useful form. Membrane based liquid-liquid extraction (LLX) is the most commonly used approach[8, 9], but LLX requires suspended solids removal from the bioreactor effluent to avoid membrane fouling. An anaerobic dynamic membrane bioreactor (AnDMBR) integrated with an LLX unit was developed to evaluate MCCAs production from food and ethanol-rich brewery waste and produce a low suspended solid effluent (permeate). The AnDMBR was equipped with stainless steel meshes that are cheaper than conventional polymeric membranes and allows the development of a biological cake layer also referred to as a “dynamic membrane” for solid-liquid separation. In terms of cost and environmental impacts, this integrated

approach is superior to other MCCAs systems that use multiple external filtration steps before the extraction unit[8, 9]. The objective of this study was to develop and evaluate the applicability of AnDMBR for integration with the downstream LLX unit for MCCAs production. Furthermore, 16S rRNA gene and 16S rRNA sequencing were employed to compare microbial community structure and activity dynamics of the suspended biomass and the dynamic membrane.

RESULTS & DISCUSSION: The AnDMBR produced a high-quality permeate (0.12 ± 0.06 g total suspended solids (TSS) L^{-1}) for an extended period of 221 days without membrane cleaning, with the lowest TSS concentration of 0.04 ± 0.01 g L^{-1} achieved on Day 69 (Figure 3). The average bioreactor TSS concentration during this period was two orders of magnitude higher (21.6 ± 9.9 g L^{-1}) than the permeate TSS. A high TSS removal efficiency of 94.6 ± 5.4% was achieved with a ratio of solid retention time to hydraulic retention time of 12.9 ± 7.4 due to high biomass retention by the dynamic membrane formation.

The permeate MCCAs concentrations were significantly higher than in the reactor (p=8.2E-05) indicating the dynamic membrane contributed to chain elongation. Consistent with this observation, the relative abundance and activity (Figure 4) of *Pseudoramibacter* and unclassified_*Clostridiales,* which have been previously associated with chain elongation[9, 10], were higher in the dynamic membrane than in the suspended biomass in the bioreactor. Biomass retention has shown to result in a higher conversion rate for MCCAs production due to increased cell density and resilience towards upsets[11, 12]. This suggests that the dynamic membrane provided conducive conditions for the enrichment of select MCCAs producers.

CONCLUSION: Dynamic membrane formation led to low permeate TSS concentration and harbored a specialized microbial community enriched in highly abundant and active MCCAs producers. The AnDMBR system is, therefore, a promising technology for the production of MCCAs along with simultaneous solids removal from solid rich streams, which is necessary for the optimal operation of the downstream extraction unit.

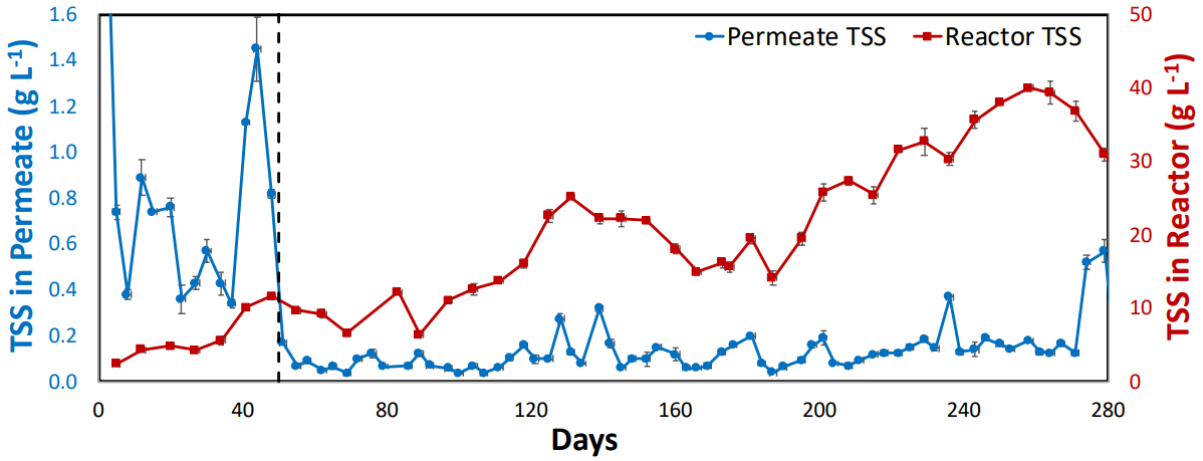

Figure 3. TSS concentration in the permeate ( ) and reactor ( ) over time. The vertical dashed line represents switch to a continuous mode of operation on Day 50.

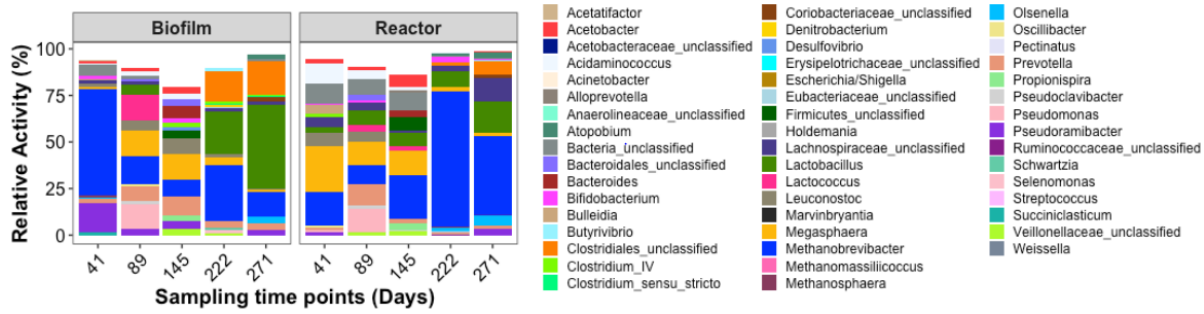

Figure 4. Relative activity of the top 15 most abundant microbial groups in each sample classified to the genus or family level in dynamic membrane and suspended biomass.

Highly selective recovery of medium chain carboxylates via anion exchange chromatography and CO2-expanded methanol desorption

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Abstract

HIGHLIGHTS:

- Carboxylates were successfully separated from a co-fermented waste stream
- Strong anion exchange resin preferentially adsorbed hexanoate and heptanoate
- Longer carboxylates were also easier desorbed, allowing their enrichment

BACKGROUND: The focus of this research was to study the recovery of a mixture of carboxylates from a complex waste stream. Different technologies for carboxylate recovery have been studied[13, 14], but high potential was presented using anion exchange chromatography for carboxylates from waste streams[15]. Recovery of longer compounds such as hexanoate and heptanoate is however less known. Some of the technologies applied up to date are membrane electrolysis[16] and anion exchange[17] for hexanoate recovery. In this study, anion exchange chromatography was used to recover a mixture of carboxylates containing hexanoate and heptanoate. This technology has been proved successful for shorter chain carboxylates such as acetate, propionate and butyrate, but its efficiency in longer carboxylates is still not well studied.

RESULTS & DISCUSSION: Municipal sewage sludge and the organic fraction of municipal solid waste were co-fermented for carboxylate production as a revalorization strategy. Hexanoate and heptanoate represented 21 and 9.5% respectively of the final composition in the effluent. The mixture of carboxylates was successfully separated from the waste stream via anion exchange chromatography. Most of the shorter carboxylates ranging from 2 to 5 carbon atoms presented a similar adsorption trend and selectivity, while valerate, hexanoate and heptanoate showed higher selectivity (Figure 5). Desorption of the compounds with CO2-expanded alcohol was also proved successful, where hexanoate and heptanoate showed a better desorption profile as well. Similar trends were observed for both the synthetic mixture and the real co-fermented sample.

The influence of bed volumes and adsorption length was also studied with the co-fermented sample. Results show that by increasing the duration of the adsorption, it is possible to desorb some of the shorter carboxylates and increase the loading of hexanoate and heptanoate in the resin.

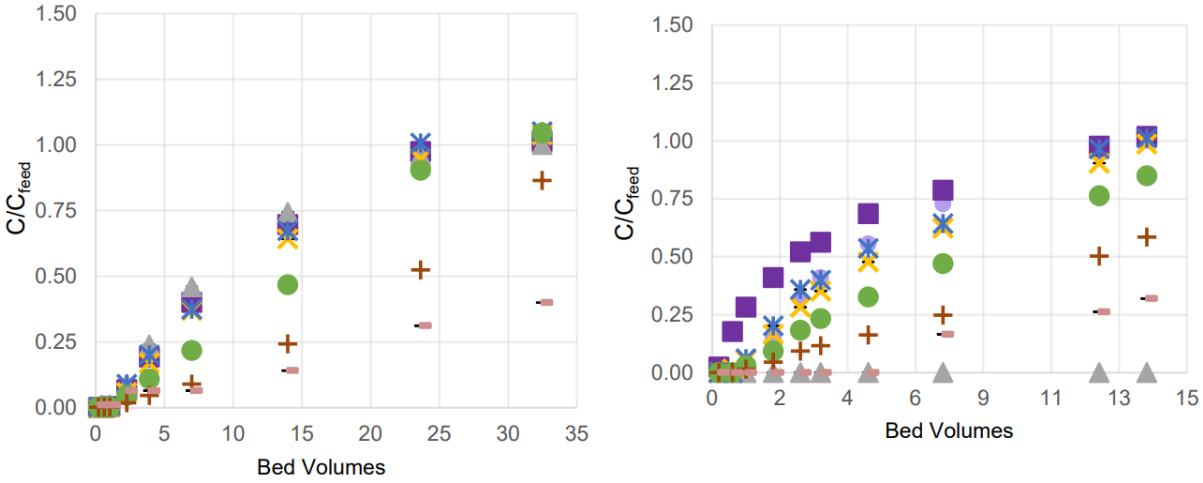

Figure 5. Breakthrough curves for a) synthetic mixture of carboxylates and b) co-fermented municipal sewage sludge and food waste (C, effluent concentration; C_{feed}, feed concentration). Average values of duplicate experiments.

CONCLUSION: This study validated the use of anion-exchange chromatography and CO_2-expanded alcohols for carboxylate recovery from waste streams. The method was more selective for medium chain carboxylates. Notably, the inorganic ions in the feed took up resin capacity and negatiely affected the process.

Bioreactor engineering and bioprocess development

Invited presentation: Resource recovery from organic waste – a short story about bioprocess development

DOI: https://doi.org/10.18174/icec2020.18006

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Optimum caproic acid production initiated from homogenised and acidified granular sludge is influenced by lactic:butyric acid ratio and concentration

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Abstract

BACKGROUND: Engineered biotechnological conversion of lactic acid to caproic acid is still in the early stages of development. A key feature of an efficient process management will be the development of a microbial community capable of efficient, stable and high-yielding conversions. In this study, the impact on inoculum development of physical and/or chemical pre-treatments of anaerobic granular sludge was evaluated. Additionally, the effect of the lactic acid and butyric acid concentration and ratio ($r_{Lac/But}$) on caproic acid yield was investigated

RESULTS & DISCUSSION: Granular sludge, homogenised in a food blender for 30 seconds before being acidified to either pH 3 or 5.5 yielded higher caproic acid concentrations than crushed or intact granules. Indeed, the pH 3, acidified and blended sludge yielded the highest caproic acid concentration. Moreover, substrate concentrations of 250 mM ($r_{Lac/But}$ = 1.5:1) and 300 mM ($r_{Lac/But}$ = 1:1) were optimal to efficiently produce caproic acid using the pH 3, acidified and blended sludge. However, when using an enriched culture, which has been

cultivated on a lactic acid/butyric acid-containing medium, the highest yields of caproic acid were achieved at a reduced substrate concentration of 200 mM ($r_{Lac/But}$ =1:1). With both sludge and enriched cultures, the lactic acid to butyric acid consumption (C rlac/but) under optimum conditions was 2:1. We report for the first time a lactic acid to butyric acid threshold concentration, below and above which the selectivity toward caproic acid is reduced. The highest caproic acid selectivity (≥ 90%) was achieved with substrate concentrations of 250 mM ($r_{Lac/But}$ = 1.5:1) and 300 mM ($r_{Lac/But}$ = 1:1) using the pH 3, acidified and blended sludge as inoculum. Caproate production was completely inhibited when the substrate concentration was increased to 400 mM ($r_{Lac/But}$ = 1:1). With these optimum substrate concentrations and ratios, the feasibility of a simplified pathway for efficient caproic acid production using lactic acid as electron donor and butyric acid as electron acceptor was demonstrated. The initial acetic acid to butyric acid elongation cycle, reported in the literature is bypassed when optimum substrate concentration and lactic acid to butyric acid ratio are used. Finally, species affiliated with *Ruminococcaceae* were likely involved in the synthesis of caproic acid.

CONCLUSION: The findings of this study have strong application potential, specifically in the design of a process that will allow for the continuous and sustainable production of caproic acid.

Characterization of carboxylate producing microbial communities

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Abstract

HIGHLIGHTS:

- Enriched for Clostridiales and Lactobacillales putatively involved in production of industrially relevant carboxylates, independent of growth feedstock.
- Alteration of operational conditions of bioreactors, e.g. increased temperature, can select for a different profile of fermentation products.
- Two recently described MCFA-producing strains, *Ca.* Weimeria bifida, and *Ca.* Pseudoramibacter fermentans[18], enriched from lignocellulosic residues, have also been identified as prominent community members in the current experiments utilizing different feedstocks.

BACKGROUND: Carboxylic acids, including succinic acid, lactic acid, and medium chain fatty acids (MCFAs), are valuable chemicals that can be produced from a variety of industrial residues by fermentative microbial communities. Residues from lignocellulosic biorefineries (conversion residue; CR), starch ethanol plants (thin stillage; TS), and the dairy industry (ultra-filtered milk permeate; UFMP) are

examples of carbon-rich, low-value co-products that are typically sent to anaerobic digesters for biogas[19] or sold as animal feed[20]. Diverse product formation from primary feedstocks can help offset operating costs, reduce the selling point of the primary products (e.g. biofuel), and ultimately make these industries more economically viable[10].

RESULTS & DISCUSSION: The CR-fed bioreactor was stable for over 100 d and produced primarily C4 and C6 fatty acids. The UFMP-fed bioreactor initially produced C6 and C8 fatty acids, and then, shifted to producing primarily butyrate (C4). TS-fed bioreactors produced a mixture of C5-C8 fatty acids. Decreasing the retention time in the TS-fed bioreactor induced a shift to succinate production. Furthermore, increasing temperature of the TS-fed bioreactor to 55°C induced shifts to lactic and propionic acid as the primary fermentation products. Identification of microorganisms using 16S rRNA gene amplicon sequencing revealed high abundance of Clostridiales and Lactobacillales in communities enriched on CR, TS, and UFMP. The UFMP bioreactor enriched for organisms related to the recently defined *Agathobacter* genus[21] within the Lachnospiraceae. In the TS reactors, *Prevotella* (*phyl.* Bacteroidetes), *Lactobacillus*-relatives, and the Clostridia *Pseudoramibacter* were prominent under MCFA-producing conditions and a disappearance of *Butyrivibrio* occurred when retention time decreased (Figure 6). The Clostridia were absent from the thermophilic TS bioreactor, and *Actetobacter* were abundant in addition to the *Lactobacillus*-relatives. Metagenomic analyses of these microbial communities is underway

CONCLUSION: The ability to enrich for carboxylate-producing taxa from the same inoculum source, but fed variety of organic-rich substrates is promising for the future of sustainable production of commodity chemicals, and is not tied to the residues of a single industry. The ability to adjust bioreactor conditions to control for certain microbial communities could help industries adapt to switches on prices or demand for chemicals produced by fermentation.

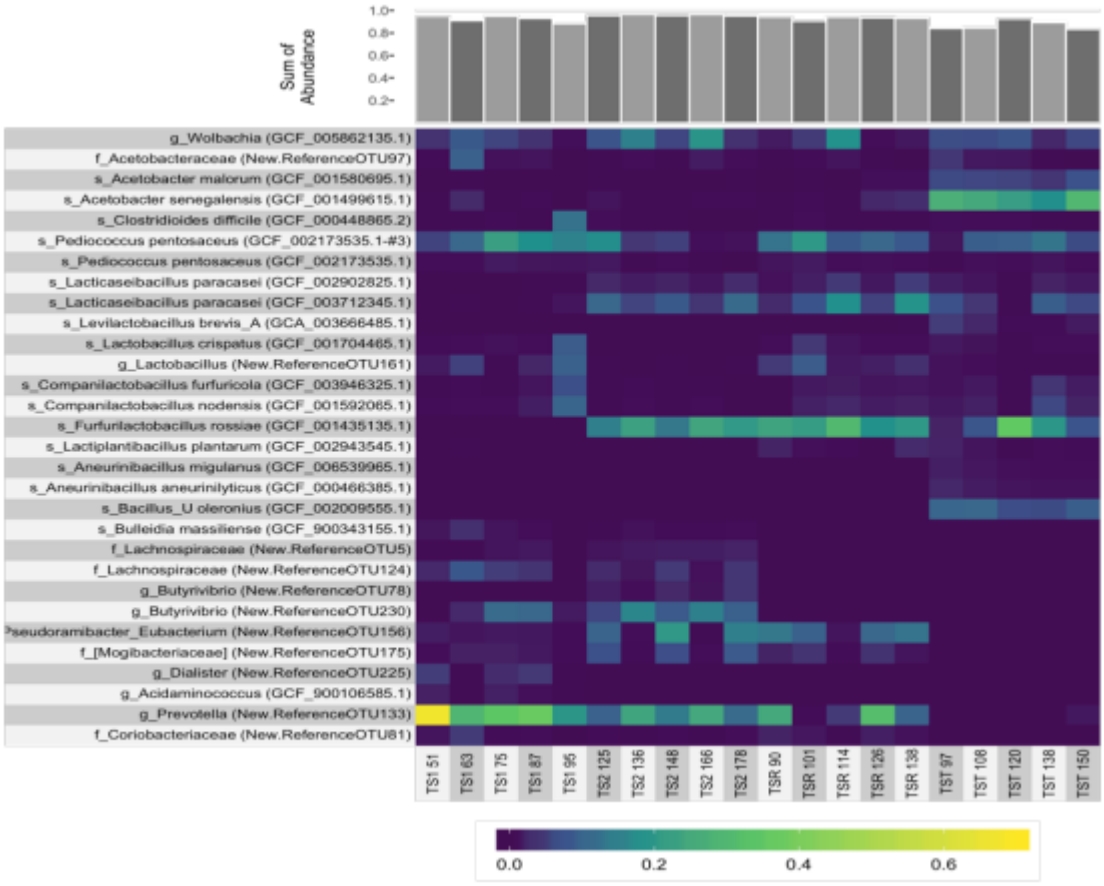

Figure 6. 16S rRNA gene amplicon based operational taxonomic units with greater than 1% relative abundance. OTUs represent microbial community during periods of stable bioreactor conditions (ca. 50 d) for four different bioreactor operational conditions. TS1, unaltered thin stillage, pH 5.5, 35°C, 6 d SRT; TS2, solids-removed thin stillage (SRTS), pH 5.5, 35°C, 6 d SRT; TSR, SRTS, pH 5.5, 35°C, 1 d SRT; TST, SRTS, pH 4.5-5.0, 55°C, 6 d SRT.

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Mildly acidic pH selects for chain elongation over propionic acid production in lactic acid fermentation

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Abstract

BACKGROUND: Lactic acid-mediated chain elongation technologies offer a highly promising route for production of medium-chain carboxylic acids (MCCA; e.g. caproic, caprylic acid). Carbohydrates can be relatively easily converted to lactic acid by – among others – *Lactobacillus* and *Olsenella*. In a second metabolic step (physically separated, or joined in one reactor stage), this lactic acid can then be elongated to caproic acid. This approach has been demonstrated repeatedly in literature[22-25], and is currently part of at least one pilot-scale approach[26]. However, nearly all reports show the persistent presence of odd-chain products, i.e. propionic acid (C3), valeric acid (C5) and heptanoic acid (C7), in the obtained product profile. Propionic acid bacteria (such as *Propionibacterium*) can convert lactic acid to a mixture of acetic and propionic acid[27], lowering product selectivity. So far, no study has explicitly investigated which parameters control

the competition between these two functional guilds. Here, we present a set of long-term bioreactor experiments, along with short-term pH-controlled batch incubations, investigating the role of pH in steering this competition. Based on pH preferences of known propionic acid producers[27] and chain elongators[28], we hypothesized that chain elongators prefer low pH, whereas propionic acid producers prefer high pH.

RESULTS & DISCUSSION: Two bioreactor communities were fed with a synthetic lactic acid medium. Initial enrichment pH were pH 5.5 (R1) and pH 5 (R2). Conversion of lactic acid was low at pH 5.5 (38.7± 18.4%), whereas pH 5.5 showed nearly complete conversion, with only transient lactic acid accumulation (Figure 7A). To test our hypothesis, pH in R2 was increased with 0.5 pH unit increments, allowing stabilisation with each increment. Product profiles at pH 5.5 (Phase II) in R2 were similar to those in R1. Further increasing pH to 6 (Phase III) did not affect caproic acid concentrations but did result in increasing propionic acid concentrations. After some operational issues (Phase IV), the reactor stabilized at pH 6.5 (Phase V), leading to a product profile made up nearly completely by acetic and propionic. To confirm this observation, pH was then decreased in the same way, in 0.5 pH unit increments. While pH 6 (Phase VI) showed a butyric acid-dominated product profile, Phases VII (pH 5.5) and VIII (pH 5) showed caproic acid dominated profiles.

Community characterization enabled us to further characterize these interactions (Figure 7B). The initial community in R2 was initially made up mostly of *Caproiciproducens*. As pH increased, propionic acid producers (*Veilonella*, *Aminobacterium*) overtook the community, mirroring the observed shifts in product profile. These communities subsequently lost terrain to *Caproiciproducens* as pH lowered again. Based on these observations, we conclude that pH is a key factor driving the interaction between chain elongating bacteria and propionic acid bacteria. We failed to completely eradicate propionic acid producers from the community and further research should investigate other approaches to control this undesirable guild.

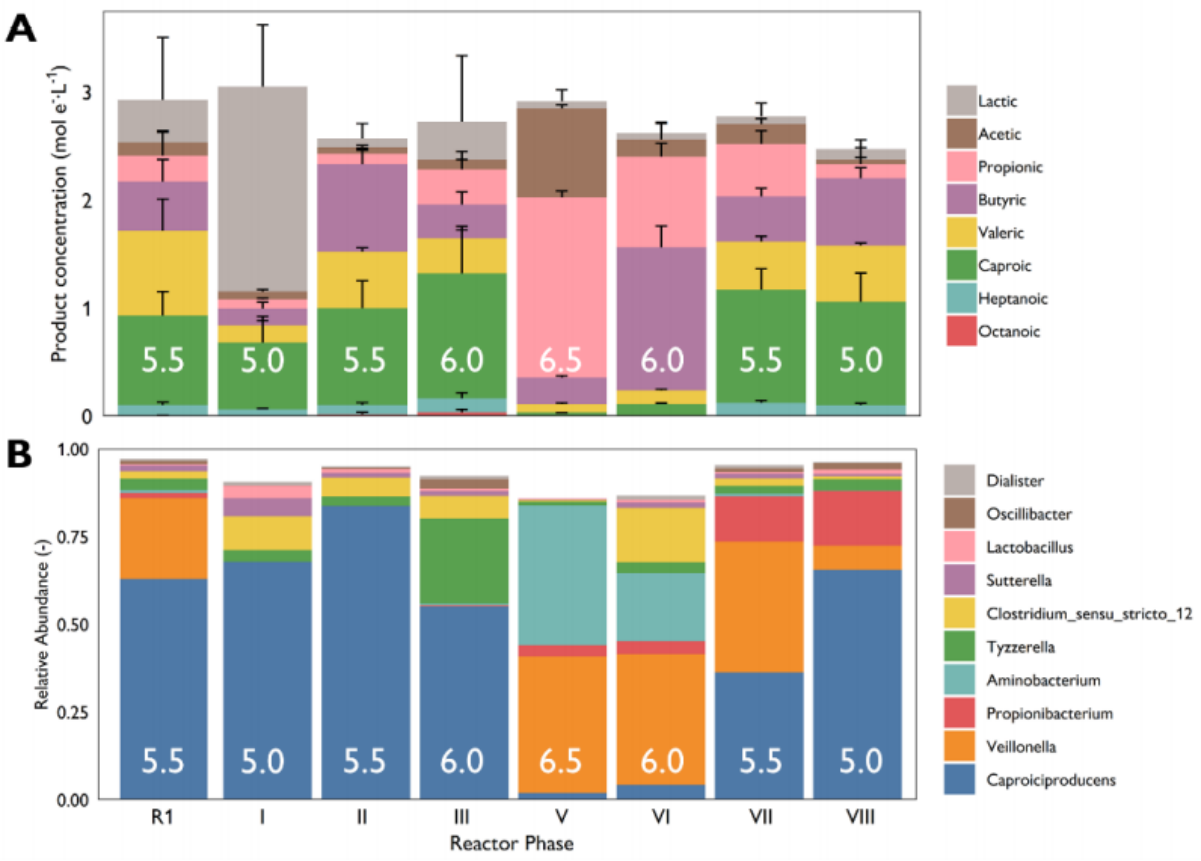

Figure 7. Product profile and community composition as a function of pH in R1 (varying pH) and R2 (constant pH 5.5).

CONCLUSION: We demonstrate here that pH is a key selecting factor during lactic acid-fed fermentations, where low pH select for chain elongating communities, whereas high pH favour propionic acid producers. This study provides a mechanistic understanding of this competitive interaction, which could enable better control of undesirable lactic acid consumption during future technology development.

H2/CO2 gas recirculation to improve lactate-to-caproate selectivity

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Abstract

HIGHLIGHTS:

- A gas recirculation process was developed to aid lactate-based chain elongation with mixed cultures
- Inhibition of methanogenesis and permanent H_2 availability improved electron flow to butyrate and caproate
- A strain 99.5% similar to *Eubacterium maltosivorans* and a *Caproiciproducens* sp. 96% similar to *Caprobacter fermentans* (16S rRNA gene) were correlated to caproate production ($p<0.05$)

BACKGROUND: Anaerobic fermentation of low value lignocellulosic biomass with mixed microbial cultures (MMC) has shown promising results for the production of high value medium-chain carboxylates (MCC) like caproate. For its feasibility as an industrial-scale process, electron donors for chain elongation cannot be procured as it is actually done in lab-scale experiments. In opposition to what is observed in pure cultures of chain-elongating bacteria, continuous presence of H_2/CO_2 in MMC has been shown to help increase the selectivity to MCC in direct and in indirect ways, possibly making supplementation of lactate, ethanol or sugars unnecessary.[29]

RESULTS & DISCUSSION: A continuous anaerobic fermenter was adapted to continuous H_2/CO_2 (80:20) recirculation with daily feed of acetate in mineral medium and weekly feed of lactate. To avoid H_2 conversion of up to 4.72 L/L/d to CH_4, a non-selective and economical methanogenesis inhibitor was used[30],

which enabled the consumption of 248 mL H_2/L/d by the MMC for conversion processes other than methanogenesis. During the methanogenesis inhibition phase (Figure 8) selectivity values of 0.165 e^- caproate/e^- lactate and 0.986 e^- butyrate/e^- lactate were achieved in comparison to 0.023 and 0.865, respectively, in the control. The enriched communities were still relatively diverse after 86 days of operation (Figure 9), and the inhibition increased abundances of the bacterial genera *Caproiciproducens*, *Eubacterium*, *Clostridium* and *Ruminiclostridium* at the cost of *Methanobacterium* and *Methanobrevibacter* (Figure 9). Regarding the two strains suspected to have produced caproate, *E. maltosivorans* is a hydrogenotroph closely related to the caproate producer *E. limosum*[31] and *Caproiciproducens* spp. and *Caprobacter fermentans* have been recently reported as caproate producers from a broad range of substrates.[32-34]

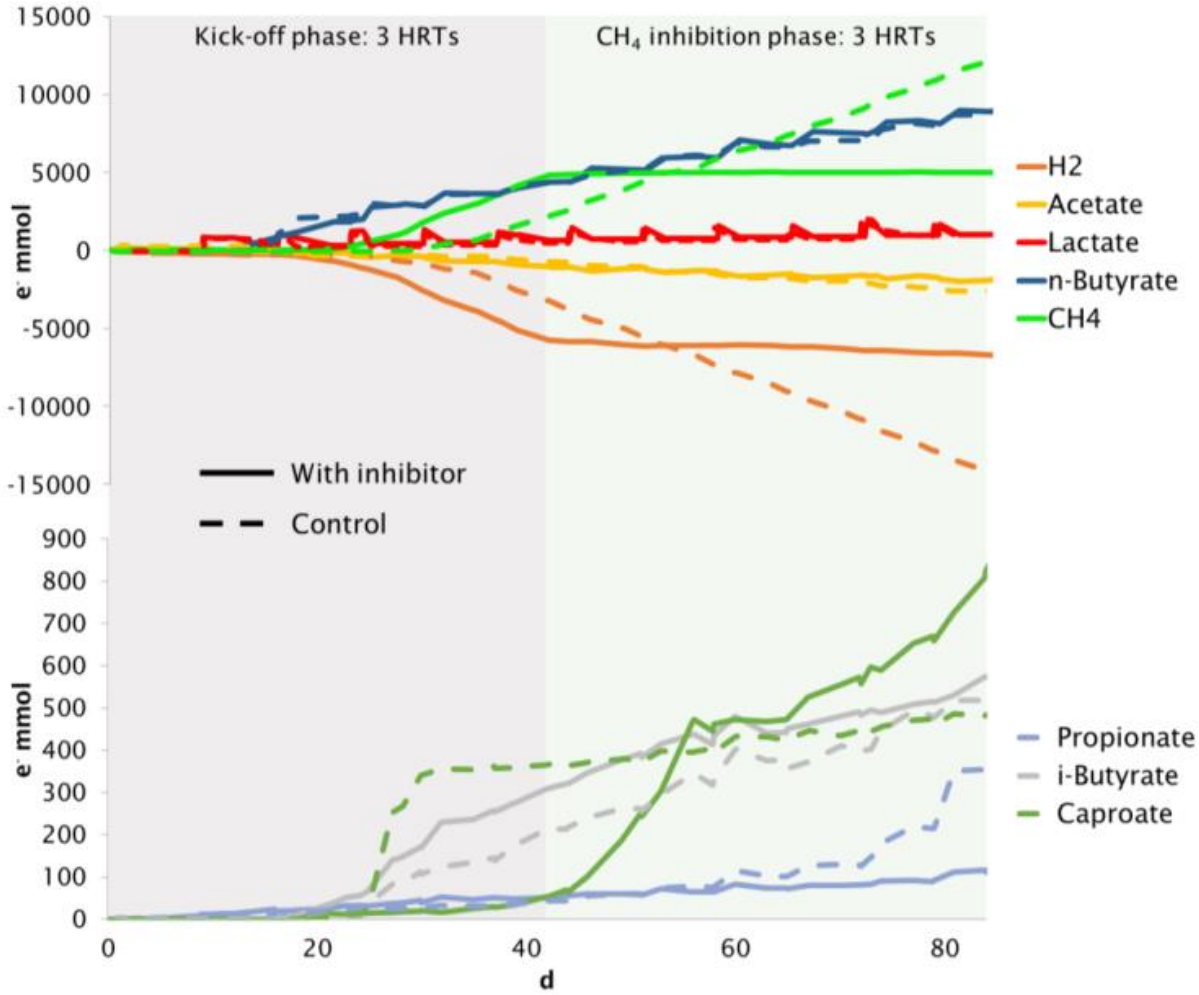

Figure 8. Accumulated electron balances for the H_2/CO_2-aided reactors.

CONCLUSION: Although caproate titers remained too low for economical extraction (up to 1.5 g/L), the gas-recirculation concept showed potential to be

used on mature chain-elongating MMC. Further molecular analyses on this system may shed light on the mechanisms that allow H_2/CO_2 to increase selectivity of conventional anaerobic fermentation to caproate.

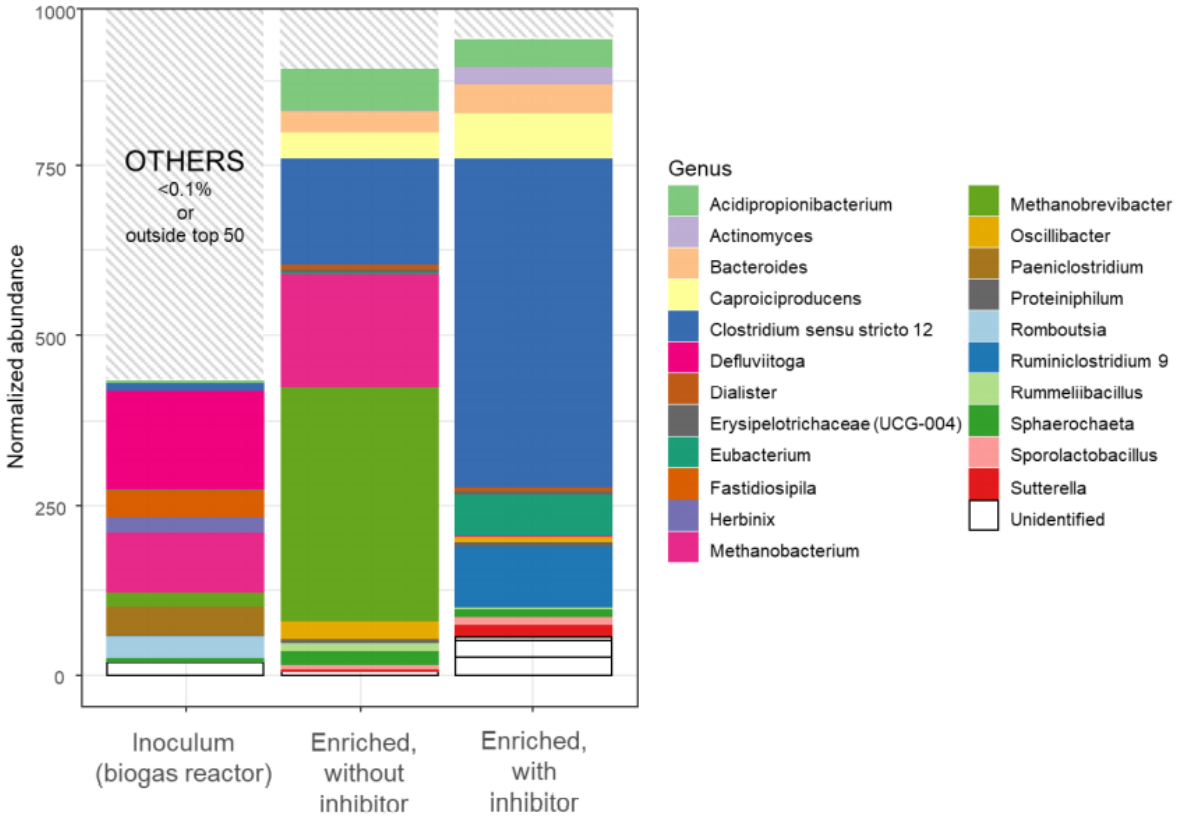

Figure 9. Community composition before and after 86 days of fermentation according to 16S rRNA amplicon sequencing.

Flow cytometry as tool to monitor chain elongation performance

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Abstract

HIGHLIGHTS:

- Flow cytometry is a rapid and reliable method to monitor the microbial community in a chain elongating reactor
- Phenotypic fingerprints of different reactor states can be differentiated via flow cytometry.
- A reactor microbiome for lactic acid chain elongation, disturbed due to caproic acid toxicity, has the potential to return to its stable state during recovery.

BACKGROUND: During the past years there has been a growing awareness that we need to strive to a circular bio economy, which has stimulated the development of novel biological processes for the production of added-value platform chemicals from organic waste streams. These new developments go hand in hand with an increasing need for process stability and performance insurance. Therefore, a good monitoring strategy is essential. The state-of-the-art mainly relies on monitoring physicochemical input and output parameters. Here we report the development of a novel monitoring strategy in which

microbial community (MC)-parameters play a key role. The MC-parameters are obtained via flow cytometry (FCM), which is a MC analysis technique that allows a fast assessment of its phenotypical diversity[35, 36]. FCM as a process monitoring and control tool has mostly been applied to the MC in environments containing little nutrients and lower microbial abundance, such as drinking water[37]. More recently, the use of FCM is emerging for the analysis of the MC and the detection of disturbances through flow cytometric fingerprinting[37, 38].

In this study, FCM is studied as a key tool for the fast detection of community changes and applied to caproic acid (CA) production via lactic acid (LA) chain elongation, with the potential of applying this on other fermentation processes in the future. We operated mixed-culture CSTR-bioreactors that were fed with a synthetic medium that contains LA (20.8 g/L) as main carbon source. During reactor operation, several physicochemical parameters (pH, electroconductivity, biogas yield and composition, carboxylate yields and spectrum, etc.) were monitored along with the microbiology. From the FCM data, the cell counts and diversity parameters were derived. The community structure was determined as a phenotypic fingerprint (PFP) based on the identification of phenotypes with a model-based approach based on Gaussian Mixture Models (GMM)[39].

RESULTS & DISCUSSION: During reactor operation, periods of decreased performance or process failure were observed. The results of one period of process failure are presented. Physiochemically, process failure was initially observed as a sudden, complete stop of the gas production, while prior to this event, around 1.2 $L_{biogas}.L_{reactor}^{-1}.d^{-1}$ containing 23.25% H_2 and 73.75% CO_2 was produced. H_2 and CO_2 are by-products of LA chain elongation[28, 40-42]. Analysis of the carboxylate spectrum showed the highest CA concentration (8.0 g/L) observed in the reactors at the moment of process failure. LA accumulated up to 8.4 g/L and the CA concentration decreased. Activity resumed once the CA-concentration had decreased to 3.2 g/L. It is therefore hypothesised that CA-toxicity was a possible cause of process failure in the process under study.

A GMM-model was generated to determine the PFP of the reactor samples. Clear shifts in the community structure based on the PFP could be observed during this period (Figure 10, a). Ordination of the samples based on the Bray-Curtis dissimilarities via Canonical Correspondence Analysis (CCA) shows the evolution of the reactor samples during normal operation, process failure and recovery

(Figure 10, b). Samples taken prior to process failure cluster together in the CCA plot, whereas the samples taken during process failure and recovery are ordinated in a counterclockwise pattern. The further in the recovery phase, the closer the samples are ordinated to the plotted to the cluster of samples from before process failure. This indicates that the microbiome performing LA chain elongation can return to its stable state prior to the crash.

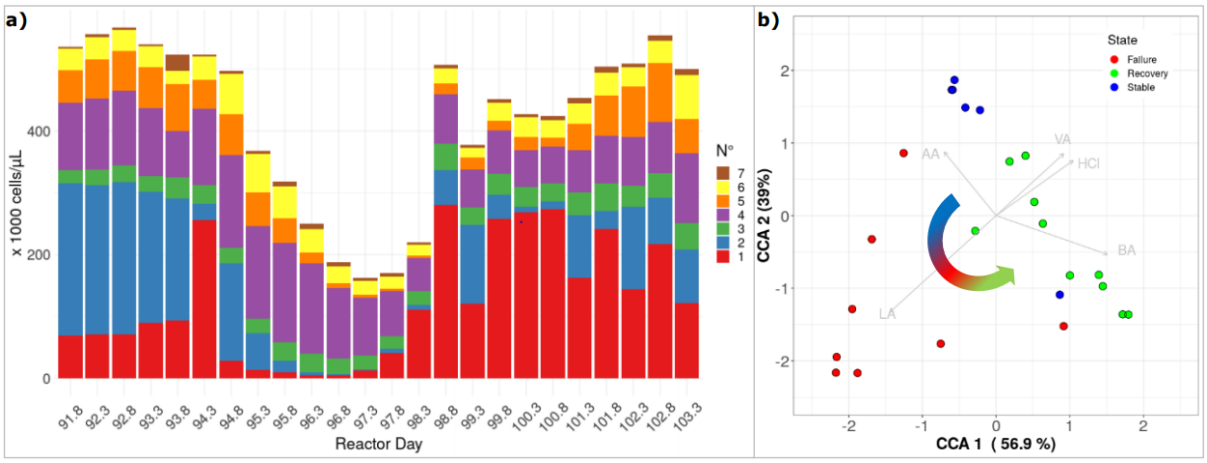

Figure 10. a) absolute phenotypic distribution over time, based on a GMM-model with seven identified phenotypes. b) CCA analysis of the samples based on the Bray-Curtis dissimilarities, constrained by the LA, acetic acid (AA), butyric acid (BA) and valeric acid (VA) concentrations, and the HCl requirements for pH control.

CONCLUSION: FCM is a suitable technique for fast determination of the PFP of a mixed-culture microbiome. Changes in reactor performance can be monitored as well on microbial level. FCM is therefore a promising tool to be included into the monitoring strategy of LA chain elongation, with the potential of extending its application on other bioproduction processes.

6x scale-up while maintaining stable production of n-caprylic acid

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Abstract

HIGHLIGHTS:

- *n*-Caprylic acid has certain advantages compared to *n*-caproic acid, including a reduced odour and a higher bactericidal activity.
- Stable production of *n*-caprylic acid was possible with a 4.2-L wet volume bioreactor that included pertraction, which is a 6x scale-up from our previous work at Cornell University[8].
- Stable production occurred at an ethanol–to–acetate substrate ratio of 6:1.

BACKGROUND:

To increase the product portfolio of microbial chain elongation, we are developing a stable bioprocess system with membrane-based liquid-liquid extraction (pertraction) to include *n*-caprylic acid (C8; *n*-octanoic acid) in addition to other medium-chain carboxylic acids (MCCAs) such as n-caproic acid (C6; *n*-hexanoic acid) and *n*-heptanoic acid (C7). Certain advantages of C8 compared to C6 exist. This includes a higher bactericidal activity, a higher heat capacity (297.9 J/K mol), a 10x lower maximum solubility concentration, and a less unpleasant odour. At Cornell University, we had already achieved a C8–to–C6 productivity ratio of more than 20:1 by feeding a mixture of ethanol and acetate into an anaerobic filter (AF) as an open-culture system[8]. However, this had been accomplished with a relatively small wet volume of 0.7 L. Here, we scaled up the process 6x to a 4.2-L wet volume AF. We investigated whether we could repeat this result from Cornell University with stable production of a considerably higher amount of C8 than C6 when a mixture of ethanol and acetate was fed as

substrate. Such a substrate mixture is present in the effluent of syngas fermentation systems.

RESULTS & DISCUSSION:

The C8-producing AF was filled with K1-filter media (Kaldnes) in a 5-L glass upflow bioreactor with an active volume of 4.2 in the presence of the filter media. The fermentation broth was recirculated continuously through a forward membrane contactor together with a solvent as part of the pertraction system. Continuous extraction of MCCAs was achieved by recirculating this solvent and an alkaline stripping solution through a backward membrane contactor. We verified the molecular structure of the produced C8 through GC/MS. In addition, we monitored the produced metabolites by GC/FID. The C8 production performance was evaluated within an experimental design by changing the substrate ratio of ethanol to acetate. When the substrate ratio of ethanol and acetate was 6:1, the C8–to–C6 productivity ratio was higher than 2.5:1. This can be seen by the ~2.5x steeper slope of the increase in the concentration of C8 compared to C6 in the alkaline stripping solution (Figure 11A), and also by the productivity ratio bar (Figure 11B). However, when the substrate ratio of ethanol to acetate was increased to 10:1, the overall C8–to–C6 productivity ratio decreased to ~1.5:1 due to lower production of C8, while the production of C6 remained constant (Figure 11B). By reversing the substrate ratio back to 6:1, we again achieved a stable production of C8, which was ~2.5x higher than C6 at a 6:1 substrate ratio of ethanol and acetate. Nevertheless, we have not been able to achieve the C8–to–C6 productivity ratio of 20:1, which we observed at Cornell University, and we are now trying to understand why.

CONCLUSION:

The bioreactor system for this study accumulated C8 up to 8000 mM C in the stripping solution (Figure 11A). We achieved a stable production of C8 at a 6:1 substrate ratio of ethanol to acetate.

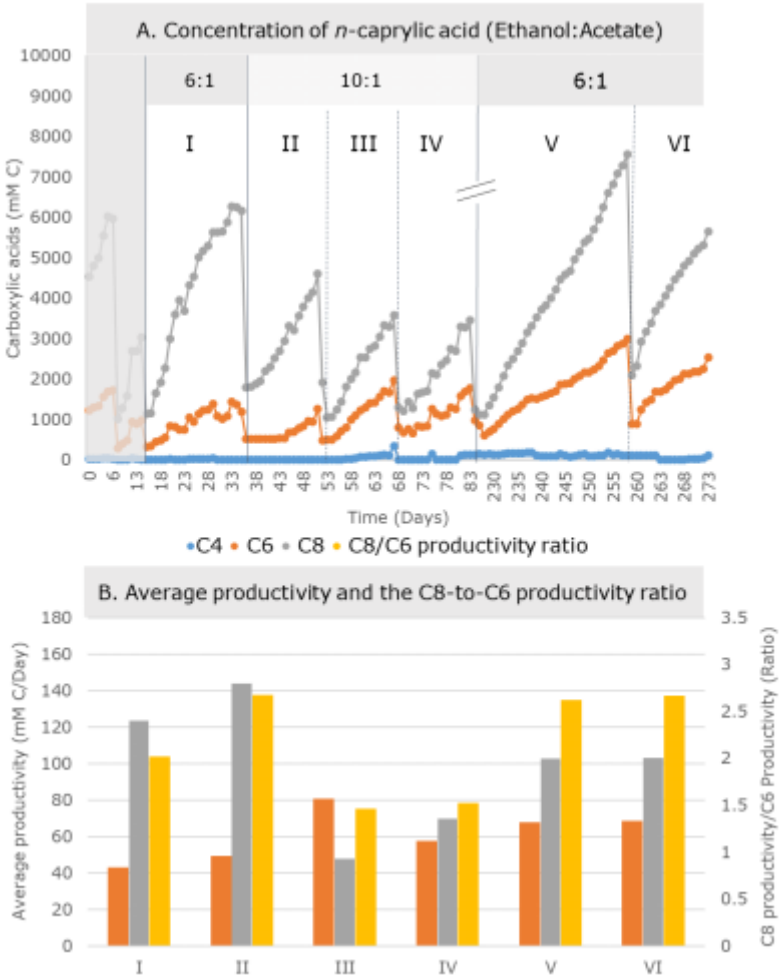

Figure 11. A. Concentration of n-caprylic acid in the alkaline stripping solution during the operating period (after each period the stripping solution was exchanged); B. Average productivity and the C8–to–C6 productivity ratio.

Invited presentation: Medium chain fatty acid (MCFA) production in a lignocellulosic biorefinery

DOI: https://doi.org/10.18174/icec2020.18012
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Continuous carbon chain elongation from one carbon compounds

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Abstract

HIGHLIGHTS:

- Multistep fermentation using microbiomes.
- Chain elongation starting from one carbon compounds is autotrophy at the thermodynamic limit
- In-line extraction allows long term continuous process

BACKGROUND: Biotechnological valorisation of organic waste streams is a promising low-cost way of producing platform chemicals that are used as precursors for bioplastics, pharmaceuticals, and fuels. The carboxylate platform is a process to produce medium chain carboxylic acids *via* microbial chain elongation. Using microbiomes for the chain elongation allows the simultaneous utilization of all pathways necessary to facilitate the multistep fermentation needed to produce the desired products.

This study tests and verifies the possibility to use one carbon compounds (*i.e.,* formic acid and methanol) as substrate, and thereby expands the scope of this platform.

RESULTS & DISCUSSION: The setup of a bioreactor system facilitating the multistep fermentation of formic acid and methanol, as well as an in-line membrane-based liquid-liquid extraction system (pertraction) to selectively remove the products, was accomplished. With this system, production rates of up to 15 mM $C*L^{-1}*d^{-1}$ (0,043 $g*L^{-1}*d^{-1}$) *n*-butyric acid and 5 mM $C*L^{-1}*d^{-1}$ (0,007 $g*L^{-1}*d^{-1}$) *n*-caproic acid were reached. We are now trying to ascertain which metabolic pathways are important and found a competition between acetogenesis, which is energetically less favourable with a Gibbs free energy

(ΔG0') of only = -111 kJ/mol, and methanogenesis, which provides a ΔG0' of -135 kJ/mol. We do not observe high concentrations of *i*-butyrate.

CONCLUSION: The continuous carbon chain elongation, using one carbon compounds to perform autotrophy at the thermodynamic limit, was proven to be viable. Preventing the accumulation of the produced medium chain carboxylic acids, and therewith associated product toxicity, the in-line extraction enables the stable long-term operation of this bioprocess. Before upscaling to a viable resource recovery system, however, we would need to ascertain whether all envisioned metabolic pathways, including chain elongation with methanol as an electron donor, are important or not.

Machine learning-assisted identification of bioindicators predicts medium-chain carboxylate production performance of an anaerobic mixed culture

DOI: https://doi.org/10.18174/icec2020.18013

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Abstract

HIGHLIGHTS:

- Productivity and yield of *n*-caproate and *n*-caprylate were enhanced by reducing the hydraulic retention time.
- Bioindicators of hydraulic retention time were inferred from 16S rRNA amplicon sequence variants (ASVs) by machine learning using a random forest approach. The recovery of metagenome-assembled genomes of these bioindicators confirmed their genetic potential to perform key steps of medium-chain carboxylate production.

- The bioindicators quantitatively predicted the productivity of *n*-caproate and *n*-caprylate with more than 90% accuracy.

BACKGROUND: The ability to quantitatively predict the chain elongation process in microbial communities provides a stepping stone to engineer microbiomes for producing desired biochemicals such as medium-chain carboxylates[43, 44]. Before engineering strategies such as changing operating conditions can be built to improve process performance, we need to analyze whether the quantifiable processes can be predicted by following microbial community dynamics. Here, we present the quantitative prediction of a lactate-based chain elongation process in bench-scale continuous bioreactors from community dynamics by machine learning. Recently, we enriched an undefined mixed culture capable of producing *n*-butyrate (C4), *n*-caproate (C6) and *n*-caprylate (C8) from xylan and lactate in a daily-fed bioreactor[7]. We found that the community developed toward predominating C4 production at the cost of C6 and C8 yields. Since C6 and C8 were the target products, it was relevant to manipulate the reactor microbiome for promoting the chain elongation process and thus optimizing C6/C8 productivity. To this end, we conducted a long-term study on the enriched chain-elongating microbiome in two parallel bioreactors continuously fed with lactate and xylan in mineral medium for 211 days.

RESULTS & DISCUSSION: We progressively reduced the hydraulic retention time (HRT) from 8 d to 2 d with different changing modes in both bioreactors. Comparing the performance at the two HRTs, the productivities at HRT of 2 d increased 2.5-fold, 5.6-fold and 7.2-fold for C4, C6 and C8, respectively; and the yields increased 1.5-fold and 2.4-fold for C6 and C8, whereas the C4 yield decreased 1.6-fold. Decreasing the HRT affected the composition and diversity of reactor microbiomes. Beta diversity analysis indicated a significant difference between the communities at HRT of 8 d and 2 d ($P < 0.001$) but no significant difference between the communities in both reactors at the same HRT ($P > 0.05$). We hypothesized that the HRT reduction induced variations in community diversity, which could be used to predict the productivity and yield of carboxylates by machine learning. Our random forest analysis consisted of two parts. First, we did the feature selection for ASVs that were relevant to community dynamics caused by HRT reduction. The two bioreactors shared 11 HRT bioindicators (ASVs). Next, we trained the random forest algorithm with

these bioindicators that were used later to predict C6/C8 productivity and yield. We reached more than 90% accuracy in the quantitative prediction of C6/C8 productivity (Figure 12). Four ASVs assigned to the genera *Olsenella*, *Lactobacillus*, *Syntrophococcus* and *Clostridium* IV were denoted as bioindicators of C6/C8 productivity. The inferred bioindicators may delineate their relevance to the enhanced C6/C8 productivity in the chain elongation process, manipulated by HRT decline. These species might be involved in the lactate-based chain elongation producing C6 and C8, e.g. species affiliated to *Clostridium* IV have been described as lactate-based chain elongation bacteria[28], while lactate formation by lactic acid bacteria (*Olsenella* and *Lactobacillus*) promoted the chain elongation process[7, 33, 45].

CONCLUSION: The quantifiable chain elongation process can be accurately predicted from 16S rRNA ASV dynamics with machine learning. If experiments with sufficient temporal and/or spatial resolution can be carried out, such a framework could be adapted for other ecosystem processes and more complex communities.

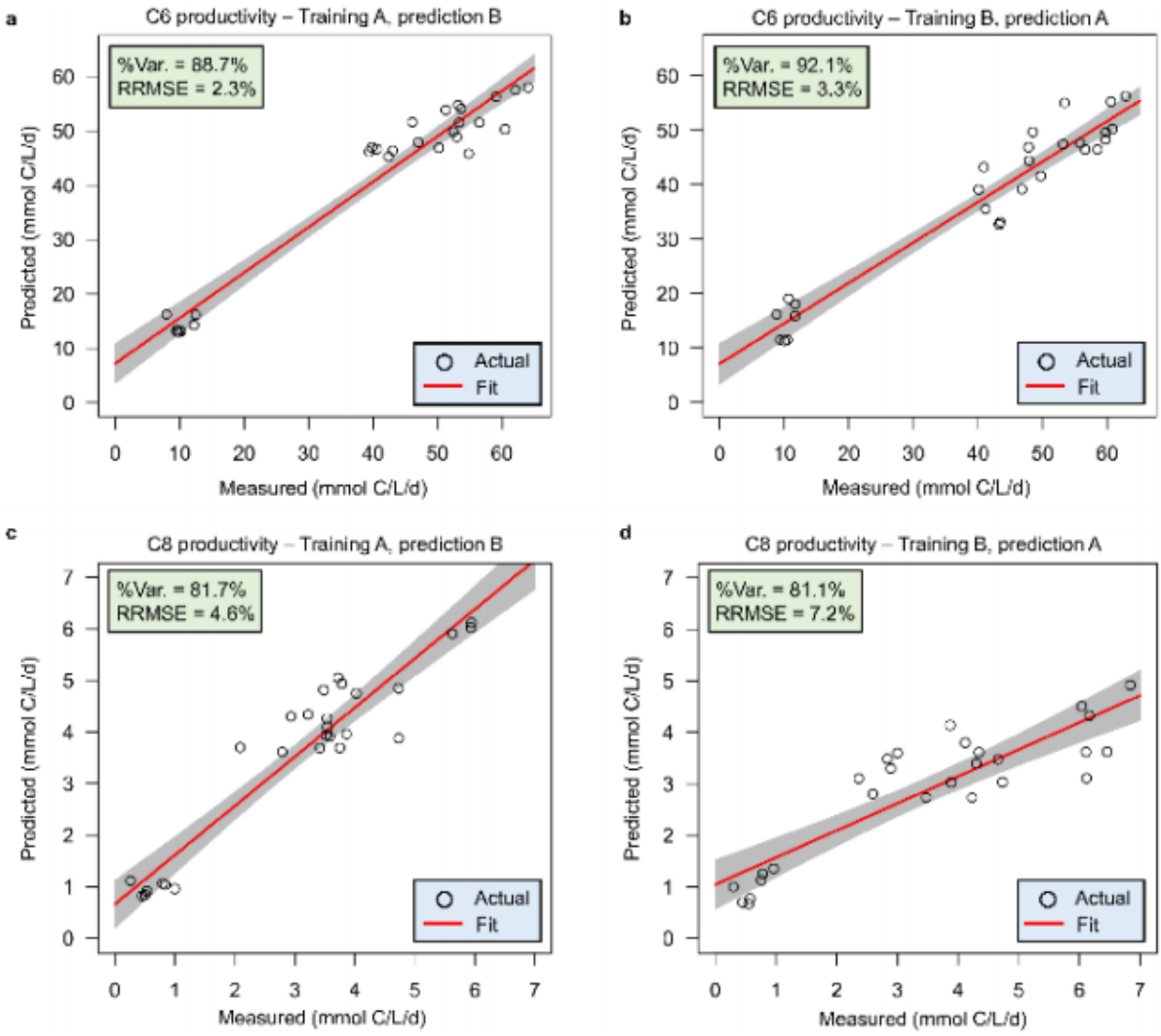

Figure 12. Prediction results of C6/C8 productivity using HRT bioindicators. a,b, Prediction performance of C6 productivity. c,d, Prediction performance of C8 productivity. In a and c, we used relative abundance data of bioreactor A as training set and relative abundance data of bioreactor B as test set. In b and d, relative abundance data of bioreactor B were used for training and of bioreactor A for testing. %Var., explained the target variance (%) of the training set. RRMSE, relative root mean square error.

Identifying chain elongation processes during the mixed-culture fermentation of proteins

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Abstract

Identifying chain elongation processes during the mixed-culture fermentation of proteins

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HIGHLIGHTS:

- Chain elongation was identified for the first time as a relevant process occurring in protein mixed-culture fermentation
- No external electron donor compound is required for the VFA elongation from proteins
- Its feasibility depends on both the chosen pH setpoint and protein composition

BACKGROUND: the chain elongation processes occurring during the fermentation of carbohydrate-rich residual streams have already been thoroughly studied, with multiple example available in literature[3]. On the contrary, little is known on the role of proteins as substrates. The only previous

studies focusing on amino acid-based chain elongation were performed with pure culture of a bacterium isolated from bovine rumen, *Eubacterium pyruvativorans*[46], which consumes short chain VFAs to elongate butyric to caproic acid, using amino acids (e.g. alanine and leucine) as electron donor compounds[47]. Conversely, the feasibility of chain elongation process in mixed-culture microbiomes fermenting proteins has not been described before.

Hence, the aim of the present study was to verify the potential of proteins as a single substrate for the acidification and condensation of longer chain carboxylates while evaluating the related mechanisms and the required operational conditions.

METHODOLOGY: integrating the results of a previous study with two different proteins, casein and gelatin[48], two fermentation batch tests were performed at pH 5 using casein as the sole carbon source. The chosen substrate-to-inoculum ratio (SIR) was of 20 g COD protein/g VSS, with macronutrients being added accordingly, while the results from the previous study were obtained with a SIR equal to 10. Acetic acid was supplemented to one of the batch tests (approx. 10 mmol/L) to better understand the role of short chain carboxylates as electron acceptor compounds in protein-based chain elongation processes.

RESULTS AND DISCUSSION: Both tests lasted 384 hours, leading to the final products spectra (mmol/L basis) observable in Figure 13. In both cases, the main products were n-butyric and iso-valeric acid (Figure 13b), while n-valeric acid production depended on whether acetic acid was supplemented at the beginning of the batch operation.

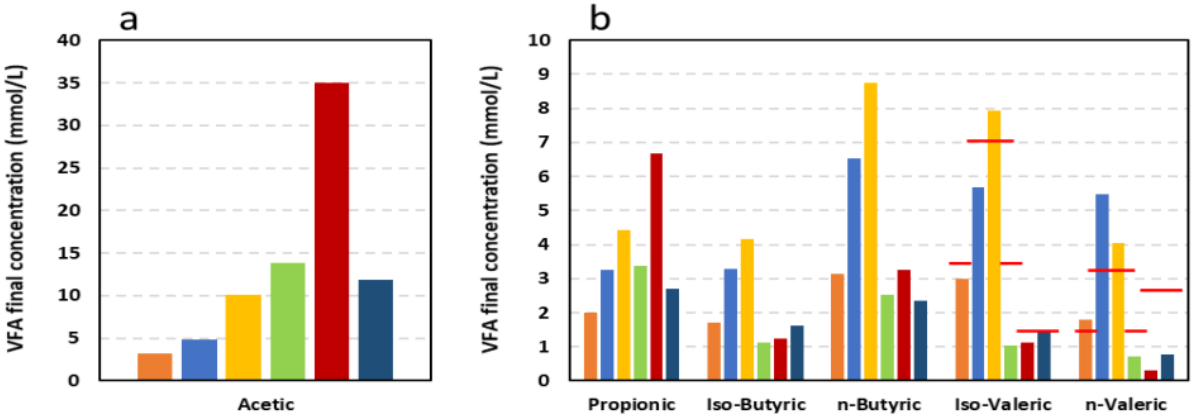

Figure 13.VFA final concentration obtained in batch fermentation tests performed with casein and gelatin at different pH values (a: acetic acid; b: remaining VFAs). ■ Casein pH 5 SIR10; ■ Casein pH 5 SIR20; ■ Casein pH 5 SIR20 with acetic acid

supplementation; ■ Casein pH 7 SIR10; ■ Gelatin pH 7 SIR10; ■ Gelatin pH 5 SIR10. The red horizontal bars illustrate the maximum theoretical production of iso and n-valeric acids based on the chosen substrate.

Consumption of acetic and propionic acid in the first stages (24-72 hours) of the casein batch tests was observed (concentration profile not shown here), suggesting the existence of chain elongation processes. Furthermore, no external electron donor supplementation was required, as amino acids probably reacted with in-situ produced VFAs. The acetic acid supplementation test helped identify the elongation process as the final concentration of this acid was comparable to the initial one. In fact, the 5 mmol/L produced in the SIR 20 test plus the 10 mmol/L of acetic acid supplemented should have led to a total 15 mmol/L at the end of this test. As only 10 mmol/L were measured (Figure 13a), it indicates that some 5 mmol/L of may have been used for chain elongation. The acetic acid supplementation also increased the overall casein conversion and diverted the condensation towards iso-valeric acid and possibly n-butyric acid. Still, protein-based fermentation appears to be especially selective towards n-valeric acid as its production was always greater than theoretically possible by acidification only in both tests (Figure 13b), given that this VFA only originates from a well identified amino acid, proline[48, 49]. Comparing these results with the ones obtained from the fermentation of different proteins[48], it appears that protein composition and pH are fundamental in determining the feasibility of the chain elongation. The process was only identified during casein fermentation at low pH (5.0); gelatin did not undergo chain elongation during its anaerobic conversion to VFAs (Figure 13b). The relative abundance of amino acids acting as electron donors might explain the differences according to the substrate.

CONCLUSION: to the best of our knowledge, chain elongation was identified for the first time during mixed-culture fermentation of proteins. This kind of process is especially attractive as it does not require electron donor supplementation as in most cases described in literature. Also, its feasibility depends on the environmental conditions (i.e. low pH) and the protein composition. This work helps to shed some light on amino acid-based chain elongation in mixed microbiomes while constituting a starting point for further studies on the subject.

Retrofitting agricultural biogas plants into biorefineries – medium-chain carboxylates production from crop silage

Authors

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Abstract

HIGHLIGHTS:

- Corn silage is a convenient substrate for achieving high product yields due to its high lactate content
- Stirred tank reactors perform better than leach-bed reactors
- pH and substrate quality affect caproate/caprylate ratio and yields

BACKGROUND: Germany has more than 9,000 biogas plants with many of them being in the agricultural sector and digesting crop silage and manure[50]. Due to recent changes in the German Renewable Energy Act, subsidies for German biogas plants will be reduced[51]. Therefore, companies are pursuing alternative business cases for the profitable operation of their plants. Complex biomass, which is used as substrate for anaerobic digestion (AD), can also be applied for the production of medium-chain carboxylates (MCC) by anaerobic fermentation (AF) including microbial chain elongation.

Corn silage is the most frequently used energy crop in AD[52]. Its high lactate concentration also predestines it for MCC production, as lactate can be utilized as electron donor for chain elongation. Only few studies with corn silage as substrate for MCC production have been done so far[45, 53]. Here, we investigated which process parameters affect the MCC production from corn silage.

RESULTS & DISCUSSION: Over the last years, we used crop silage in different ways as substrate in lab-scale AF reactors of up to 15 L volume for MCC production. In 100 mL batch experiments with corn silage inoculated from an MCC producing reactor, much lower MCC yields were obtained than in the semi-continuous reactors with the same substrate (Table 1). According to this, small-scale batch experiments were not comparable to bigger reactor systems, meaning batch experiments cannot be used determine the production potential of such substrate.

As automatic pH control is beneficial for high MCC production, tank reactors with a powerful stirring system were superior to leach-bed reactors, due to their direct pH measurement and a faster and more thorough mixing of pH controlling chemicals with the fermentation broth. At a pH of 5.5, maximum caproate titers of 6.12 g L^{-1} were observed. Caproate production was related to lactate-based chain elongation as lactate contained in the silage and that produced during AF was completely consumed[45]. In contrast, caprylate was produced in considerable amounts of up to 1.83 g L^{-1} only at higher pH above 6.0. This higher pH range simultaneously led to consumption of ethanol produced from the substrate. Therefore, it can be assumed that chain elongation with ethanol might be involved in caprylate production[45].

Table 1. Carboxylate yields in g $kgVS^{-1}$ in batch and semi-continuous AF.

Product	Batch AF	Semi-continuous AF
C2-C4	95 - 138	258 ± 7
n-Caproate (C6)	11 - 32	90 ± 2
n-Caprylate (C8)	0.9 - 4	26 ± 1

From all process parameters, substrate quality affected the MCC production most. High quality corn silage contains high concentrations of lactate and acetate, whereas fatty acids such as propionate and butyrate are contained in low concentrations. With low quality silage, less lactate can be provided for chain elongation resulting in low MCC production. By supplementing substrate with lactate for simulating good quality, MCC production could be improved, however, high quality corn silage could not be completely replaced (Figure 14). This indicated that a good corn silage is characterized by more than high lactate concentration.

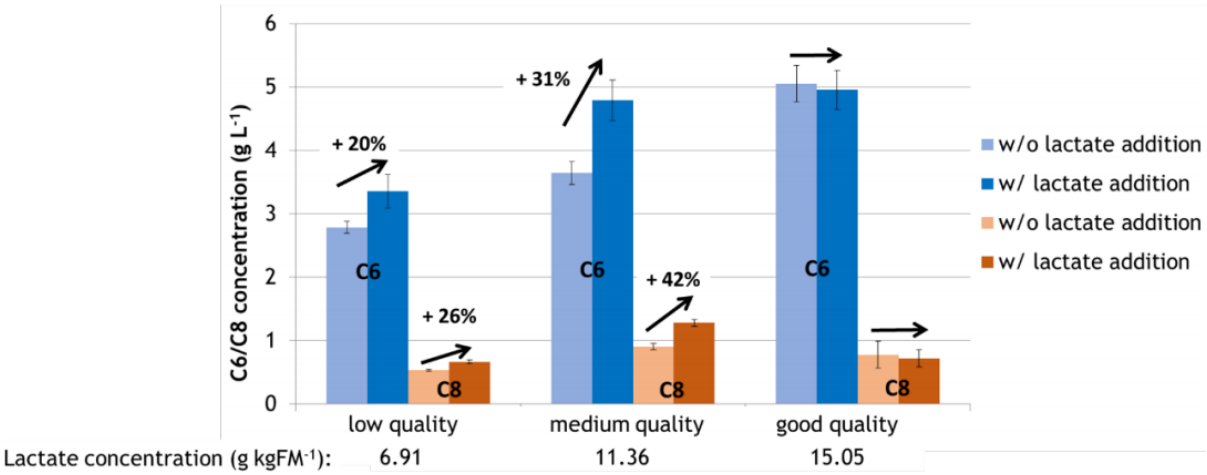

Figure 14. Caproate and caprylate production from different quality corn silage in stirred tank reactors. In all experiments with lactate addition, equal lactate amounts were supplied including lactate from the substrate.

CONCLUSION: Agricultural biogas plants digesting crop silage can be retrofitted to biorefineries by inserting an MCC production step and enabling a combined material and energetic utilization of biomass. Thus, biomass can be used in a more sustainable way due to the formation of value-added products. Thus, new perspectives can be opened up for biogas plant companies. The product spectrum of AF can be controlled to a certain extent via the pH value. Plant operators should pay much attention to high substrate quality as it affects the AF even more than the AD process.

Poster presentation: Medium-chain carboxylic acids production using consortia from winery wastewater, ruminal fluid and granular sludge

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Abstract

BACKGROUND: Chain elongation is an anaerobic fermentation process that produces medium-chain fatty acids (MCFA) –such as caproic acid– from volatile fatty acids (VFA) and ethanol. MCFA have a high added value of up to 10 times more than ethanol and up to 5 times more than methane. The inoculum source is significant when a robust consortium needs to be obtained for the MCFA production. Adequate syntrophic interactions could increase the productivity of the MCFA and offer economic viability to the process. This work evaluated the potential of an endogenous consortium from winery wastewaters to produce MCFA (white and red wine manufactured at Querétaro, Mexico). The process performances were compared to other inocula, one harvested from a ruminal fluid (sheep slaughterhouse) and granular anaerobic sludge (flour wastewater treatment). The native winery wastewaters consortium has been exposed to high ethanol concentrations (100 g/L). That could favor not only a faster MCFA production process but also the production of acids with longer carbon chains where higher concentrations of ethanol are required.

RESULTS & DISCUSSION: The highest production of caproic acid was 5.8 g/L using an endogenous winery wastewater microbiota. However, when the ruminal fluid was used as inoculum, Caprylic acid (2.8 g/L) was produced in

addition to caproate (3.5 g/L) and heptanoic acid (2.1 g/L). Caprylic acid is a medium-chain carboxylic acid with higher added value compared to caproic acid. The use of granular sludge reveals the production of only caproic acid (3.7 g/L). Although with all the inoculums caproic acid was obtained, faster production rates were observed with the endogenous consortia of winery wastewaters. That can be explained because the microorganisms were already adapted to elevated ethanol concentrations. The other two inocula required more time to adapt to ethanol (500 mmol). Microbial community analyses indicated that the operational taxonomic unit (OTU) associated with *Clostridia* (85%) and *Bacteroides* were dominant and positively correlated with elevated MCFA productivities. Results also suggested that the microbiome evolved in such a way that the MCFA production was improved.

CONCLUSION: It was evidenced that the highest MCFA production was obtained with an endogenous consortium from winery wastewaters. Higher productivity of caproic acid was observed compared to the other inocula used in this work. Nevertheless, caprylic acid was produced with ruminal fluid. The microbial community analyses indicated that OTUs for *Bacteroides* spp. and *Clostridium* spp. were positively correlated with the MCCA production.

Poster presentation: Effect of domestication and microbial community on medium chain fatty acids production from Chinese liquor distillers' grain

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Abstract

Effect of domestication and microbial community on medium chain fatty acids production from Chinese liquor distillers' grain

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HIGHLIGHTS:

- Pit mud is a suitable inoculum for MCFA prodction from residue
- Domestication of pit mud improves caproate production
- *Caproiciproducens* and *Lactobacillus* coexist in pit mud help CE

BACKGROUND: Chinese strong-aroma type liquor used food crops as substrate, and in the later stage of fermentation, relied on the pit mud for the esterification reaction of ethanol with caproate, butyrate and acetate to produce the aforementioned flavour substances. Chinese liquor distillers' grain (CLDG) is an incomplete fermentation byproduct, which contained polysaccharides, and has a huge output. For the chain elongation (CE) process, pit mud is an appropriate inoculum rich in caproate-producing microbiome.

In this study, CLDG was used as fermentation substrates, pit mud and domesticated one were used as inoculum to produce MCFAs with complex substrates was investigated; the fermentation effects and microbial community changes were studied.

RESULTS & DISCUSSION:

The caproate concentration inoculated by shallow pit mud and the domesticated one were 394 and 449 mg COD/g VS (substrate) respectively. And the caproate carbon selectivity increased from 32.5% to 37.1% in total fatty acid yield. The abundance of caproate producing bacteria was improved. The domestication shallow pit mud makes more butyrate participated in the CE process and converts more caproate. The concentration of lactate and ethanol during fermentation showed shallow pit mud can effectively utilize lactate to produce caproate.

The characteristics of microbial composition in shallow pit mud and domesticated one showed the rich *Caproiciproducens* and *Lactobacillus* coexisted, and their abundance has increased from 12% to 25% and 3.7% to 18%, respectively. The strain of *Caproiciproducens* spp in the pit mud produce caproate by metabolizing lactate (Figure 15).

CONCLUSION: Using the pit mud of CSAL as inoculum and CLDG as substrate, the shallow pit mud fermentation system produced caproate. By domestication of shallow pit mud, the caproate concentration and the caproate carbon selectivity increased. The analysis of microbiome showed that lactate in CLDG was used as the main ED, and the main caproate producing species was *Caproiciproducens* spp.

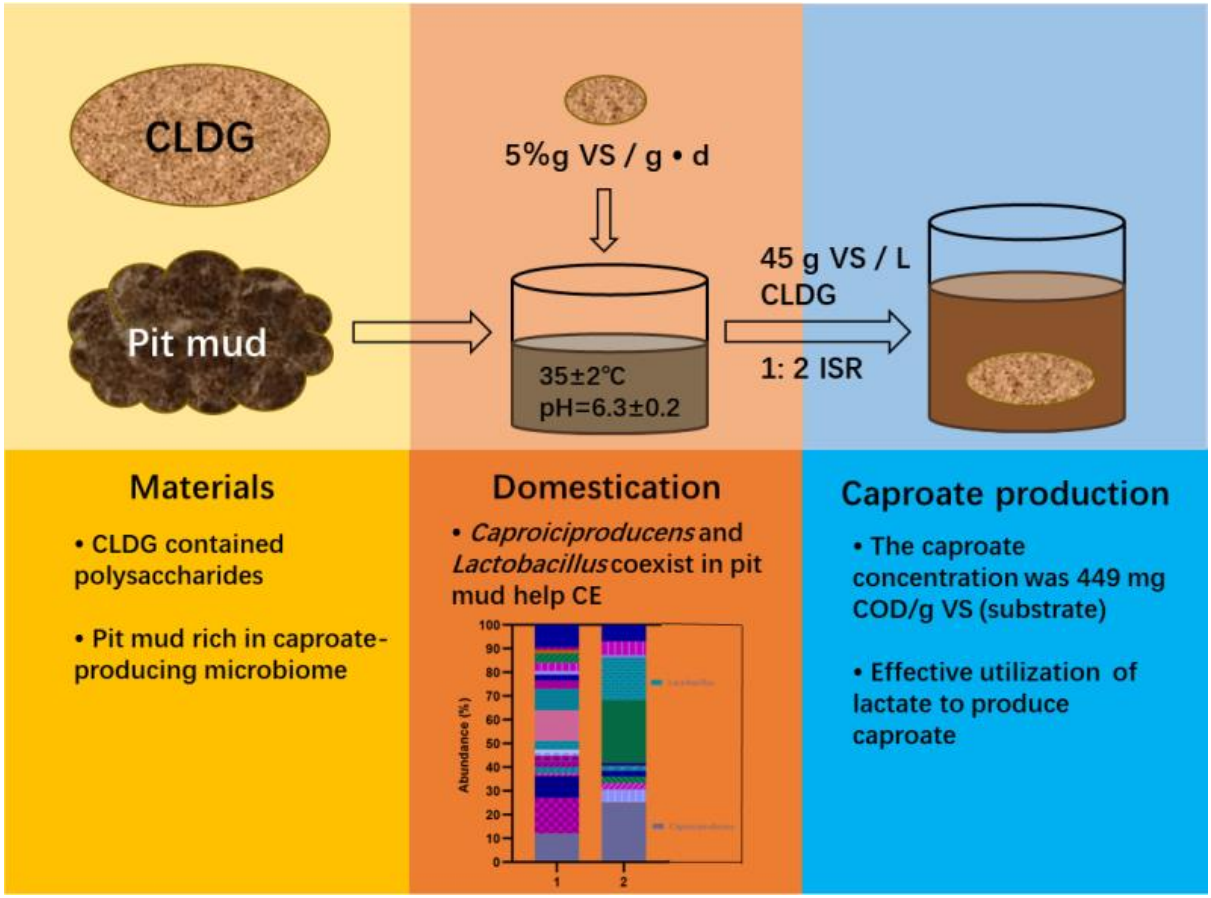

Figure 15. Graphical abstract.

Key-note presentation: Carboxylate platform; Conversion of biomass to chemicals and fuels

DOI: https://doi.org/10.18174/icec2020.18018
Prof. Mark Holtzapple - Department of Chemical Engineering; Texas A&M University, USA

HIGHLIGHTS:

- The carboxylate platform allows any biodegradable biomass to be converted to valuable hydrocarbon fuels and industrial chemicals
- Yield and conversion are enhanced by co-fermenting gases (CO_2 and H_2)
- Co-treatment and product extraction improve yields

BACKGROUND: Through primary fermentation, a consortium of marine microorganisms converts biodegradable biomass to acetic, propionic, and butyric acids, as well as ethanol and lactic acid. Methane is suppressed using methanogen inhibitors (iodoform). Through chain elongation, the primary products are converted to higher acids up through octanoic acid (C8). The mixed acids are recovered via extraction and are chemically processed to hydrocarbon fuels and industrial chemicals.

RESULTS & DISCUSSION: Depending upon the scale and feedstock cost, hydrocarbon fuels derived from the carboxylate platform can compete with crude oil priced at $42 to $98/bbl. Utilizing wastes as feedstock greatly lowers production costs.

The first commercial plant, which produces high-value carboxylic acids from food waste, is currently being constructed.

CONCLUSION: Currently, despite low oil prices and the nascent state of technology development, waste biomass can be economically converted to high-value chemical products in small “boutique” plants. Eventually, chemical markets will saturate and wastes will be converted to fuels. When fossil fuel use reduces because of declining reserves or a carbon tax, energy crops will be grown to supply fuels.[54]

Key-note presentation: Production of carboxylates by microbial co-cultures growing on syngas

Prof. Diana Sousa – Microbiology; Wageningen University, the Netherlands

Microbiology, pathways, informatics, genetics

Revamping the model CO-fermenting acetogen, Clostridium autoethanogenum, as a CO2-valorisation platform

DOI: https://doi.org/10.18174/icec2020.18019

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Abstract

BACKGROUND: Acetogenic bacteria can convert waste gases into fuels and chemicals. There is potential to build on the success of commercial gas fermentation towards bacterial artificial-photosynthesis, wherein renewable substrates could serve as facilitators of CO_2-valorization[55-58]. Steady state quantification of carbon flows greatly enhances cyclical-development of CO_2-utilizing bioprocesses.

RESULTS & DISCUSSION: CO_2 and H_2 chemostats had limitations, namely growth rate and stability (Figure 16A). However, the fermentation revealed that captured carbon (460 ± 80 mmol/gDCW/day) was significantly distributed to ethanol (54 ± 3 C-mol% with a 2.4 ± 0.3 g/L titer; Figure 16B-D). Quantification of the fermentation enabled flux balance analysis and comparison to previous datasets[59]. This indicated CO-supplementation may lessen a potential constraint resulting from limited reduced ferredoxin at the pyruvate:ferredoxin oxidoreductase. Supplementation with a small amount of CO enabled co-utilisation with CO_2, and enhanced CO_2 fermentation performance significantly (9.7 ± 0.4 g/L ethanol with a 66 ± 2 C-mol% distribution, and 540 ± 20 mmol CO_2/gDCW/day; Figure 16).

CONCLUSION: We established a dataset quantifying steady-state of the model acetogen *C. autoethanogenum* during autotrophic-CO_2/H_2 growth in chemostat cultures. This enabled analysis *via* FBA, and highlighted CO as a potential supplement. CO supplementation successfully improved metabolic stability and CO_2 utilization. This was the first time that intracellular fluxes for net uptake of CO_2 (with enhancement) where characterized. Industry is actively developing gas fermentation to valorize CO_2[56, 58]. Previously, genetic and process engineering of gas fermentation successfully developed the technology for industrial CO valorization[60]. Therefore, progression to industrial CO_2 valorization is foreseeable, and CO supplementation may play a role in the continuing diversification of industrial gas fermentation.

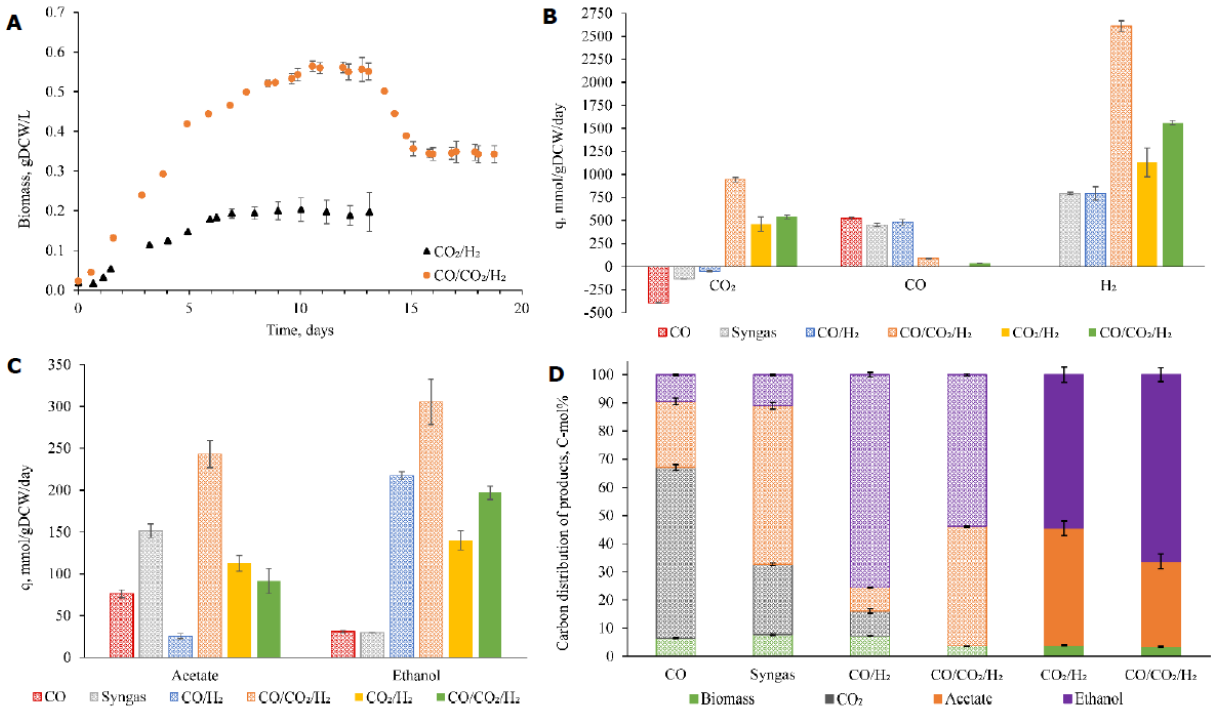

Figure 16. Important fermentation characteristics of Clostridium autoethanogenum *in autotrophic chemostats. Growth curves of novel fermentations with standard deviation at steady-state (**A**) – following thirteen days of fermentation CO/CO_2/H_2 chemostats where switched to a dilution rate (D) of 1 day^{-1}. Specific rates of uptake (**B**) and production (**C**) for important metabolites. Product carbon balances (**D**). Results from Valgepea et al. (2018) are also displayed (**B**, **C** & **D**). Values represent the average ± standard deviation between biological replicates. Number of biological replicates, and detailed gas composition for each fermentation are available in Table 1. Patterned bars indicate a D of 1 day^{-1}, full bars indicate a D of 0.5 day^{-1} (**B**, **C** & **D**). Abbreviations:* q *–specific rate,* DCW *– dry cell weight.*

Genomic and metabolic features of three novel Clostridia isolates involved in lactate-based chain elongation

Authors

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Abstract

HIGHLIGHTS:

- Three novel species of chain elongation bacteria were isolated that convert lactate to *n*-caproate, *n*-butyrate and *iso*-butyrate.
- Their complete genomes were assembled using a short-read (Illumina) and long-read (Oxford Nanopore) sequencing approach.
- The genomes encode all pathways involved in lactate oxidation to acetyl-CoA, reverse β-oxidation, H_2 formation and energy conservation (Rnf and Ech complexes).

BACKGROUND: Hitherto, few bacterial species have been reported to convert lactate to *n*-caproate. Recently we reported reactor microbiota that produce *n*-caproate from corn silage by anaerobic fermentation[45]. To identify and characterize the key players of this bioprocess, we isolated anaerobic fermenting bacteria on lactate as sole carbon source and analysed their metabolic profiles as well as the genetic background of lactate-based chain elongation (CE).

RESULTS & DISCUSSION: After >700 days of enrichment on lactate in mineral medium, various pure strains with CE function were isolated. Three caproate-producing isolates designated BL-3, BL-4 and BL-6 represented new species based on their 16S rRNA gene sequences[61] and were selected for whole genome sequencing. Hybrid *de novo* genome assembly based on short and long reads resulted in circular genomes (Figure 17) that encode all pathways for acetyl-CoA formation from lactate, reverse β-oxidation, H_2 formation and energy

conservation (Figure 18). For ferredoxin re-oxidation, strains BL-3 and BL-4 employ the Rnf complex while strain BL-6 has the Ech complex. Surprisingly, no butyryl-CoA:isobutyryl-CoA mutase was detected, which would be expected for the formation of iso-butyrate. Phylogenomic analysis based on average nucleotide identity (ANI) revealed that the strains are only distantly related to the next relative species (BL-3: 83.9% ANI to *Clostridium luticellarii*; BL-4: 73.6% ANI to *Clostridium jeddahense*; BL-6: 68.6% ANI to *Ruminococcaceae* bacterium CPB6).

CONCLUSION: The three novel isolates broaden our view on the microbial diversity of chain-elongating *Clostridia* and represent valuable model systems for further studies on lactate-based CE.

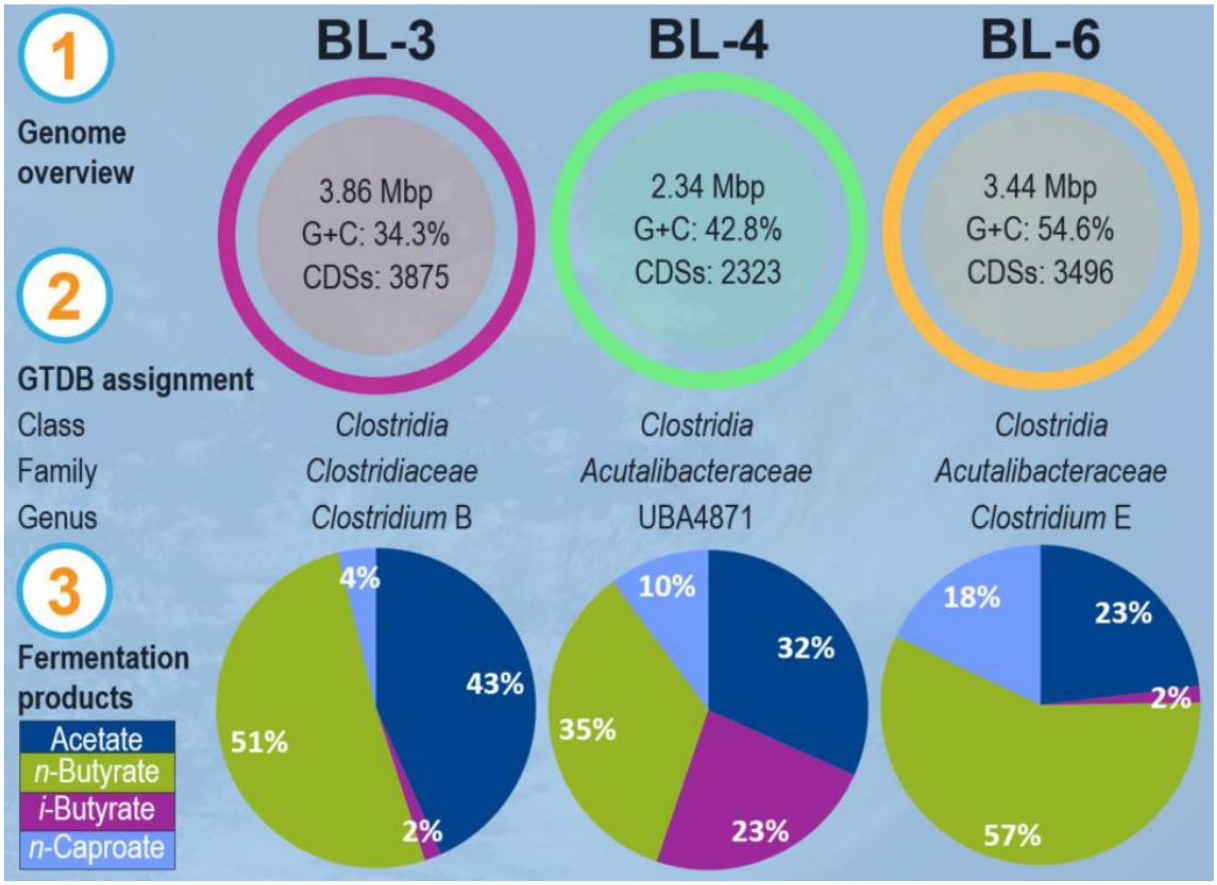

Figure 17. Genome overview, taxonomic affiliation and fermentation profiles (C mM ratio) of the three strains isolated on lactate as sole carbon source.

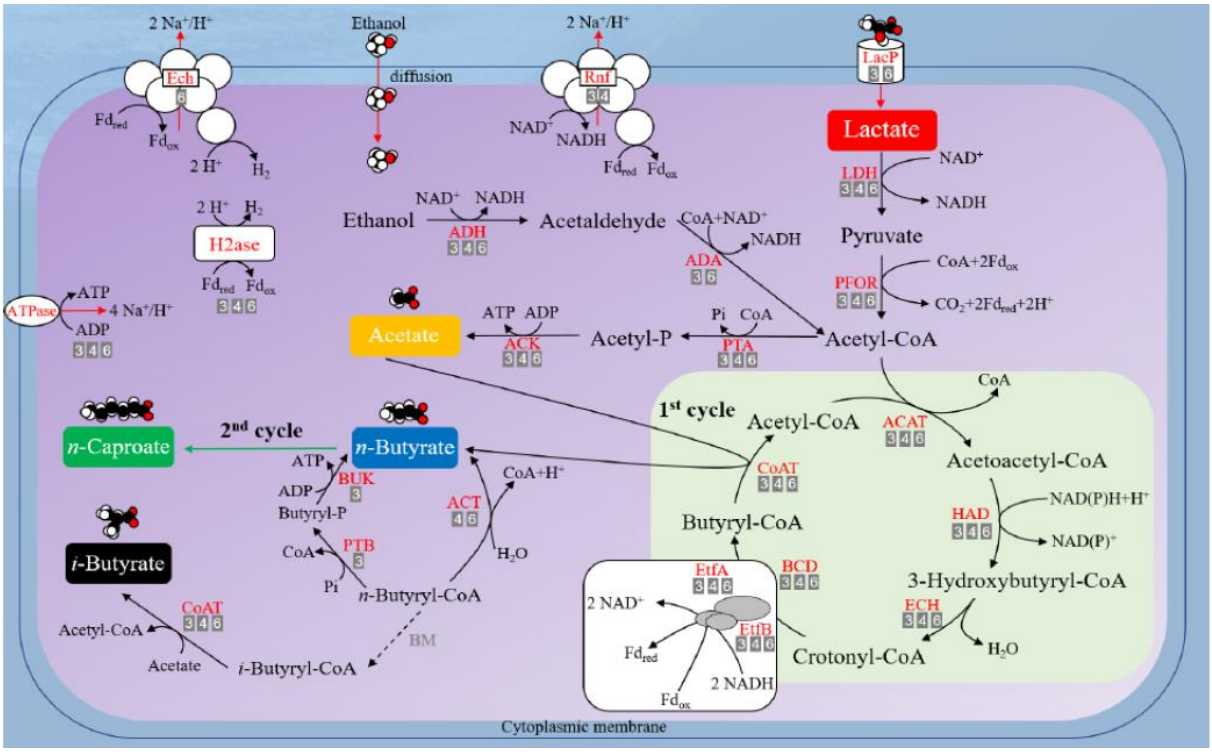

Figure 18. Metabolic pathways identified in the three genomes

The isolate Caproiciproducens sp. 7D4C2 produces n-caproate at mildly acidic conditions from hexoses: genome and rBOX comparison with related strains and chain-elongating bacteria

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Abstract

BACKGROUND: Bulk production of medium-chain carboxylates (MCCs) with 6-12 carbon atoms is of great interest to biotechnology. Open cultures (*e.g.*, reactor microbiomes) have been utilized to generate MCCs in bioreactors. When in-line MCC extraction and prevention of product inhibition is required, the bioreactors have been operated at mildly acidic pH (5.0-5.5). However, model chain-elongating bacteria grow optimally at neutral pH values.

RESULTS & DISCUSSION: We isolated a chain-elongating bacterium (strain 7D4C2) that thrives at mildly acidic pH. We studied its metabolism and compared its whole genome and the reverse β-oxidation (rBOX) genes to other bacteria. Strain 7D4C2 produces lactate, acetate, *n*-butyrate, *n*-caproate,

biomass, and H_2/CO_2 from hexoses. With only fructose as substrate (pH 5.5), the maximum *n*-caproate specificity (*i.e.*, products *per* other carboxylates produced) was 60.9 ± 1.5%. However, this was considerably higher at 83.1 ± 0.44% when both fructose and *n*-butyrate (electron acceptor) were combined as a substrate. A comparison of serum bottles with fructose and *n*-butyrate with an increasing pH value from 4.5 to 9.0 showed a decreasing *n*-caproate specificity from ~92% at mildly acidic pH (pH 4.5-5.0) to ~24% at alkaline pH (pH 9.0). Moreover, when carboxylates were extracted from the broth (undissociated *n*-caproic acid was ~0.3 mM), the *n*-caproate selectivity (*i.e.*, product *per* substrate fed) was 42.6 ± 19.0% higher compared to serum bottles without extraction. Based on the 16S rRNA gene sequence, strain 7D4C2 is most closely related to the isolates *Caproicibacter fermentans* (99.5%) and *Caproiciproducens galactitolivorans* (94.7%), which are chain-elongating bacteria that are also capable of lactate production. Whole-genome analyses indicate that strain 7D4C2, *C. fermentans*, and *C. galactitolivorans* belong to the same genus of *Caproiciproducens*. Their rBOX genes are conserved and located next to each other, forming a gene cluster, which is different than for other chain-elongating bacteria such as *Megasphaera* spp (Figure 19).

CONCLUSION: *Caproiciproducens* spp., comprising strain 7D4C2, *C. fermentans*, *C. galactitolivorans*, and several unclassified strains, are chain-elongating bacteria that encode a highly conserved rBOX gene cluster. *Caproiciproducens* sp. 7D4C2 (DSM 110548) was studied here to understand *n*-caproate production better at mildly acidic pH within microbiomes and has the additional potential as a pure-culture production strain to convert sugars into *n*-caproate.

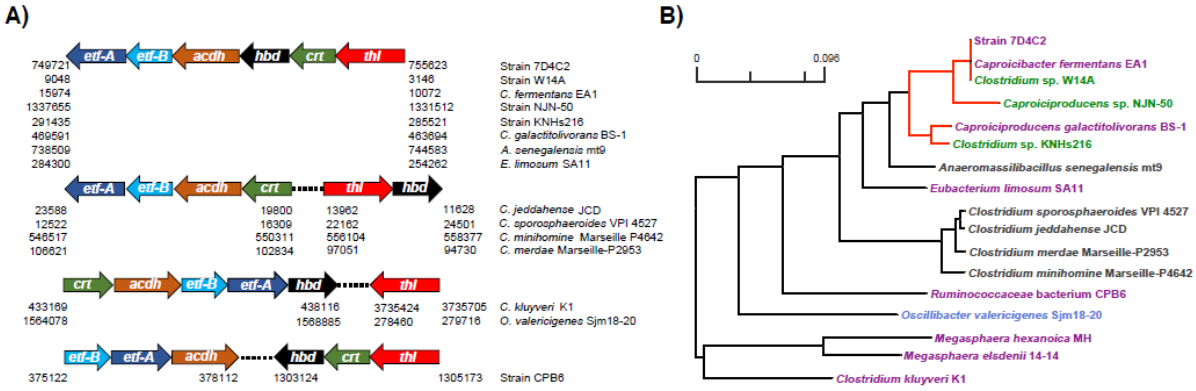

Figure 19. rBOX genes for strain 7D4C2 and bacteria with similar genes, as well as in known n-caproate producers: A) position of the rBOX genes that cluster together in these bacteria. The numbers below the arrows indicate

the position (base pairs) of the genes for each bacterium on the right column; and B) consensus phylogenetic tree of all 6 rBOX genes that cluster together. Red lines indicate the* Caproiciproducens *clade. Microbial names highlighted in purple denote* n-*caproate producers, in green are potential* n*caproate producers, and in blue* n-*valerate producers. *As the rBOX genes in the* Megasphaera *species do not cluster, for this analysis, we considered the genes most similar to strain 7D4C2.*

Methanol-based chain elongation of acetate to i/n- butyrate at thermodynamic equilibrium of isomerization by an enriched microbiome

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Abstract

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HIGHLIGHTS:

- An enriched methanol-based chain elongation microbiome was capable of producing isobutyrate and n-butyrate at a concentration ratio that approached thermodynamic equilibrium of isomerization.

The usage of isobutyrate as electron acceptor within an enriched ethanol-based chain elongation microbiome led to isocaproate formation. Similarly isovalerate addition led to isoheptanoate formation, however at a much lower selectivity.

- Carboxylates larger than acetate can be reduced to their corresponding alcohols with electrons derived from ethanol oxidation towards acetate. To

drive this process the reactant to product ratios of the coupled reaction need to be sufficiently high.

BACKGROUND: Microbial chain elongation can be employed to convert organic residues into platform chemicals such as carboxylates and alcohols. Sometimes trace amounts of branched carboxylates are observed, but until now no branched chain elongation process was developed. We investigated how to enrich microbiomes to form branched carboxylates and thereby expand the product spectrum of microbial chain elongation and its application range. In earlier research it was shown that isobutyrate could be formed using a microbiome that performed methanol-based chain elongation[62]. Toxicity of high butyrate concentrations was suggested as one of the causes for isomerization. A hypothesis was formulated that by lowering the pH and thereby aggravating the butyrate toxicity, isobutyrate formation from acetate and methanol could be stimulated. A long term continuous reactor experiment was operated at various pH levels to enrich the microbiome towards production of n-butyrate and isobutyrate or mainly n-butyrate[63].

In the realm of ethanol-based chain elongation, it has extensively been shown that odd-chains (e.g. n-propionate and n-valerate) can be elongated by two carbon atoms at a time[64]. It was hypothesized that isobutyrate could be elongated to isocaproate in a similar fashion. We verified this hypothesis via a long-term continuous reactor experiment[65]. Additionally, we performed experiments to increase the selectivity of the discovered branched chain elongation. This led to more insights into alcohol formation during chain elongation.

RESULTS & DISCUSSION:

We showed with the methanol reactor experiment that the selectivity for i-C_4 and/or n-C_4 could be reversibly adjusted by operating at different reactor pH values. A reactor pH of 6.75 led to formation of (carbon per total carbon of products) 0.78 n-C_4 and 0.024 i-C_4, whereas a reactor pH of 5.2 led to a selectivity of 0.24 n-C_4 and 0.65 i-C_4. A microbial community analysis showed that a *Eubacterium* genus was responsible for the formation of n-C_4, whereas a *Clostridium luticellarii* strain was responsible for the formation of a mixture of i-C_4 and n-C_4. At low pH (5.2-5.5) the isobutyrate and n-butyrate concentration

ratios approached thermodynamic equilibrium of isomerization. The highest achieved volumetric productivity for isobutyrate was 2.4 ±0.3 g L^{-1}.

The ethanol reactor experiment showed that isocaproate (4-methyl pentanoate, i-C_6) can be produced via ethanol based chain elongation of isobutyrate. The enriched microbiome was dominated by *Clostridium kluyveri* and formed isocaproate from isobutyrate and ethanol at a rate of 1.4 ± 0.1 g L^{-1} day^{-1}. This amounted to 20% of all formed compounds based on carbon atoms. The presence of other electron acceptors, besides isobutyrate, strongly reduced the selectivity of isocaproate formation; there was a strong preference for straight chain elongation. Because acetate elongation towards straight chains is competing with branched chain elongation for ethanol, reducing the available acetate was one of the strategies to increase selectivity for isocaproate formation. However, when acetate was low, while ethanol and larger carboxylates were abundantly available, this stimulated reduction of the larger carboxylates to their corresponding alcohols. Electrons for this reduction seemed to originate from ethanol oxidation. The effect was augmented when the CO_2 supply became limiting for hydrogenotrophic methanogenesis, allowing the reducing equivalents to be channelled towards formation of the alcohols instead.

CONCLUSION:

A microbiome was successfully enriched to perform methanol-based chain elongation of acetate to form both n-butyrate and isobutyrate (at pH 5.2 and 5.5). Further we show that ethanol-based chain elongation can used to elongate isobutyrate towards isocaproate and that alcohol formation can be stimulated during acetate and CO_2 limited operation. These results provide the pioneering basis to further develop new products from open culture chain elongation fermentation systems.

Invited presentation: Multi-omics study on chain elongation processes for medium chain carboxylic acids

DOI: https://doi.org/10.18174/icec2020.18021

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Production of isobutyric acid from methanol by Clostridium Luticellarii

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Abstract

Production of isobutyric acid from methanol by *Clostridium Luticellarii*

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HIGHLIGHTS:

- *Clostridium luticellarii* can produce isobutyric acid from methanol

Supplementation of acetic and butyric acid as electron acceptors enhanced isobutyric acid titer, selectivity and productivity

Maximum isobutyric acid production was achieved at pH 6.50

BACKGROUND: The urgency to mitigate climate change has triggered the development of strategies to reduce CO_2 emissions, including microbial technologies to convert CO_2 into multi-carbon products[66, 67]. CO_2 fermentation is hampered by low gas-liquid mass transfer due to the low solubility of H_2[68, 69]. CO_2-derived methanol can serve as alternative feedstock, circumventing the solubility issue[69, 70]. Some acetogens can use methanol as electron donor, with a product spectrum dominated by acetic and butyric acid[70, 71]. The product portfolio of methanol fermentation has recently been expanded by the

observation that isobutyric acid (iC4) was produced by mixed culture (2.0 $g.L^{-1}.d^{-1}$)[62]. A recent study of the ecology of a similar system revealed that the microbiome was dominated by *Eubacterium* and *Clostridium* spp., and that isobutyric acid production was closely linked to the abundance of the *Clostridium* sp.[72]. This study isolated the organism responsible for isobutyric acid production, explored its capacity to produce isomers from a broad range of substrates and investigated potential metabolic-triggers to isomerisation, such as pH and electron acceptor availability. [RG1]

RESULTS & DISCUSSION: Here we obtained seven isolates that exhibited iC4 production ranging from 2.22 $g.L^{-1}$ to 3.90 $g.L^{-1}$ from an in-house isobutyric acid-producing CSTR. The 16S rRNA gene sequence analysis of the isobutyric acid-producing isolates revealed that they all shared high similarity with *C. luticellarii* DSM 29923.

C. luticellarii DSM 29923 ability to produce iC4 from different carbon sources (i.e. glucose, glycerol, methanol, ethanol, lactic acid and CO2 & H_2) was screened. Growth was supported on all carbon sources except ethanol, and *C. luticellarii* produced iC4 only when grown on methanol and acetic acid (1.51 ± 0.07 $g.L^{-1}$) and CO_2 & H_2 (0.12 ± 0.01 $g.L^{-1}$). Subsequently, *C. luticellarii* ability to produce other isocarboxylic acids was explored with methanol (200 mM) as electron donor in combination with different carboxylic acids as electron acceptors. The production of other isocarboxylic acids was not detected under the tested conditions. However, *C. luticellarii* was shown to be able to synthesise valeric and caproic acid with acetic and propionic as electron acceptor, respectively. Here, we also screened the effect of pH on iC4 production by *C. luticellarii*. Over the range of pH 5.50 to 6.50, increasing pH led to higher cell densities and iC4 production (from 0.37 ± 0.02 $g.L^{-1}$ to 3.91 ± 0.06 $g.L^{-1}$). Further increasing the pH up to 7.00 resulted in higher iC4 selectivity (83% at pH 7.00), but the methanol converted was halved.

Finally, the evolution of the product spectrum was monitored throughout the batch incubation under three selected conditions (M, MA and MAB, Figure 20). Acetic and butyric acid production started immediately during growth on methanol with CO_2 as only available electron acceptor (M). iC4 production only started after six days when *C. luticellarii* started consuming the acetic acid

produced in-situ. When acetic acid was fed as an electron acceptor in condition MA, butyric and iC4 were directly coproduced. Under the condition with butyric acid supplementation (MAB), only iC4 net production occurred during the first six days. Following day six, butyric acid started to accumulate. Overall, it was observed that the supplementation of acetic and butyric acid also enhanced iC4 production in terms of production rate (from 0.120 ± 0.024 $g.L^{-1}.d^{-1}$ in condition M to 0.420 ± 0.012 $g.L^{-1}.d^{-1}$ in condition MAB). Additionally, condition MA revealed that preliminary accumulation of butyric acid is not necessary to trigger isobutyric acid production.[RG2]

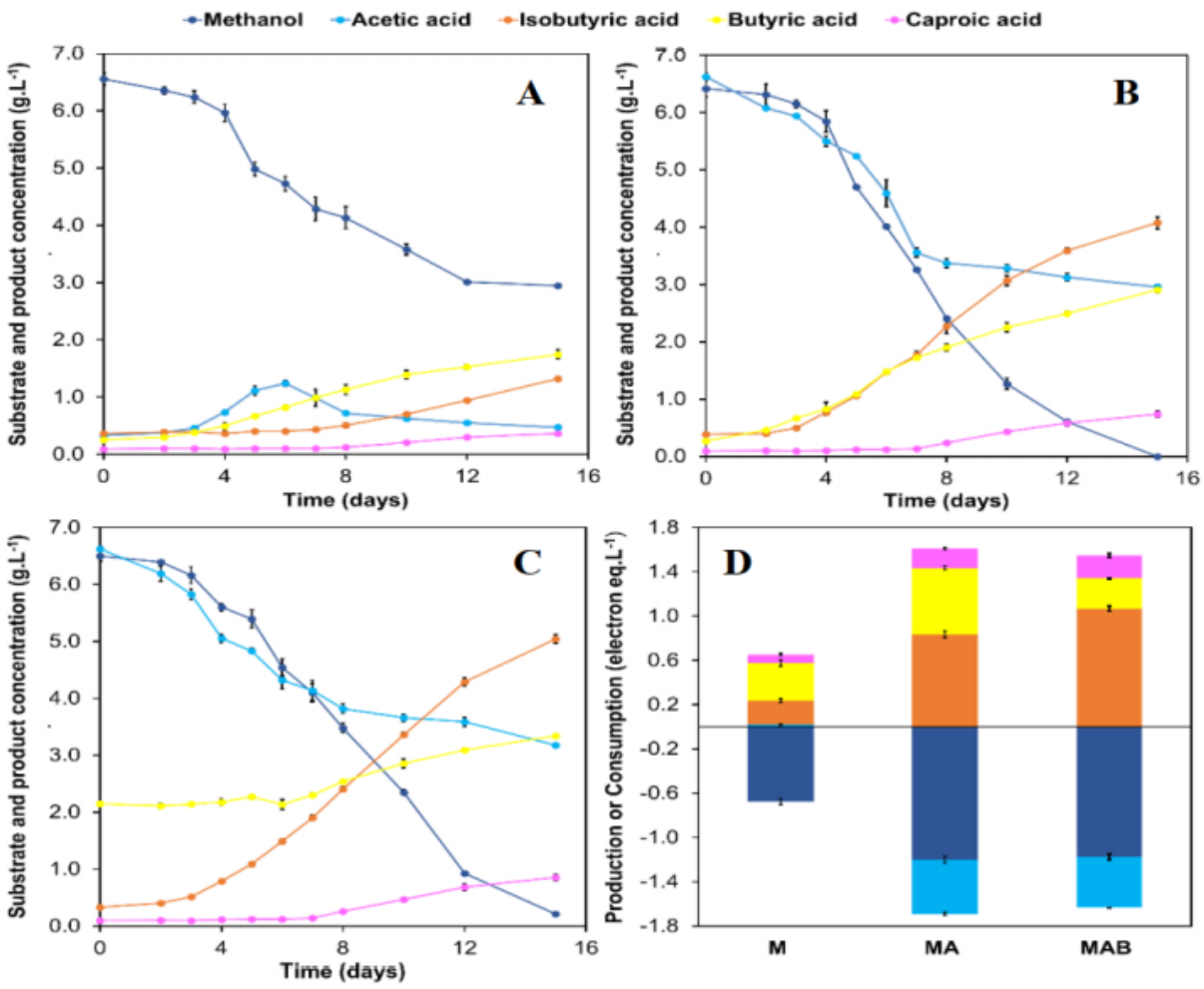

Figure 20. Substrate and product concentration profile during batch growth of C. luticellarii *DSM 29923 on methanol (Panel A; M), and methanol and acetic acid with (Panel C; MAB) and without (Panel B; MA) butyric acid supplementation. Panel D represents the electron balance.*

CONCLUSION: *Clostridium luticellarii* can produce isobutyric acid from C1 carbon sources, including methanol. It can generate isobutyric acid without the external addition of acetic and butyric acid as electron acceptors, although their presence steered isobutyric acid production in terms of final titer, selectivity, and

productivity[RG3] (maximum of 5.04 ± 0.08 g.L-1, 70% and 0.420 ± 0.012 g.L-1.d-1, respectively). pH was shown to significantly influence isobutyric acid production with the highest production obtained at pH 6.50.

The occurrence and ecology of microbial chain elongation of carboxylates in soils

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Abstract

BACKGROUND: Work in the 1930s with freshwater and marine muds as inocula produced several isolates of a novel species, *Clostridium kluyveri*, the model microorganism for chain elongation. Since the isolation of *C. kluyveri*, work on microbial chain elongation has developed based on two trajectories. Early research focused on microbiological characterizations and resolving the biochemical pathways involved in producing and oxidizing fatty acids in *C. kluyveri*. A more recent focus exploits chain elongation by *C. kluyveri*-containing complex microbial communities or other members of *Firmicutes* for converting streams rich in organic compounds (i.e., food waste, municipal solid waste) to medium-chain carboxylates.

Like municipal or agriculture waste streams, anaerobic soils and sediments often contain an abundance of biodegradable organic compounds. While chain elongation is not known to occur in soils, the conditions required for chain elongation do exist in natural anaerobic soil environments. For example, transient fermentative accumulation of short-chain carboxylates and simple alcohols has been detected in anaerobic soils up to mM concentrations. In top soils, short-chain carboxylates are also exudates from the roots of plants. Given that microorganisms capable of chain elongation utilize carboxylates and simple alcohols as substrates, we hypothesized that microbial chain elongation could be a likely metabolic pathway in anaerobic soils.

RESULTS & DISCUSSION: In this study, we utilized two top and two deep, biogeochemically-diverse soils in microcosms and enrichments in an effort to understand the occurrence of microbial chain elongation in soil and its involved

microbial ecology. The microcosms consisted of 25 g of soil and 75 mL of mineral medium. The microcosms were amended with chain elongation substrates at concentrations used in biotechnology-focused studies (e.g., 100 mM acetate + 100 mM ethanol, 100 mM ethanol, 100 mM acetate + H_2) or at environmentally relevant concentrations observed during fermentative events in soil (e.g., 2.5 mM acetate + 2.5 mM ethanol). All microcosms were incubated in the dark at 30 ℃ for 14 days. Select soil microcosms conditions were subjected to enrichment in semi-batch cycles. Each cycle was typically incubated for 1-2 weeks and four to five semi-batch cycles were completed per soil enrichment.

All soils showed evidence of microbial chain elongation activity in one or more conditions within several days of incubation. Microbial chain elongation of acetate and ethanol was the major metabolism occurring in three of the soils which produced butyrate, butanol, and hexanoate. 53-104% of the consumed millielectron equivalents from added substrates were recovered as C4-C6 carboxylates and alcohols by the end of the 14-d incubation in the soils microcosms. The combination of acetate and ethanol at environmentally relevant concentrations triggered the use of acetate as a soil electron acceptor in chain elongation and produced butyrate and ultimately hexanoate. In microcosms without added substrates, acetate was the only metabolite detected on day 14 of incubation at concentrations similar to those recorded in anaerobic incubations of forest, bog, or patty soils. Enrichment in semi-batch cycles maintained high rates of conversion of added substrates to butyrate and hexanoate. In one of the soil enrichments, octanoate became the main product from chain elongation after 3 semi-batch cycles. The microbial ecology developed in enrichments presented similarities to communities from bioreactors, rumen, and human gut microbiome. *C. kluyveri,* but also several *Firmicutes* genera not known to undergo chain elongation, were substantially enriched under chain-elongating conditions.

CONCLUSION: Our work brings evidence that top and deep soils harbor a readily-active metabolic potential for chain elongation of carboxylates. Chain elongation occurred at high and low (environmentally relevant) soil concentrations of acetate and ethanol, typical metabolites from organic compound fermentation. We expect chain elongation to be triggered in soils during events of high release of carboxylates and reduced electron donors

(ethanol, H_2) from animal, plant, and microbial decay. While the extent and role of microbial chain elongation within the anaerobic food web in soils remains to be determined, we propose it contributes to banking electrons and carbon from labile substrates into higher-carbon compounds. Microbial chain elongation could be a mechanism to decrease the chances of acetoclastic methanogenesis and to overall delay mineralization of organic compounds within a soil.

Simulating chain elongation with constraint-based metabolic modelling

DOI: https://doi.org/10.18174/icec2020.18023

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Abstract

HIGHLIGHTS:

- We reconstructed the metabolism of six functional guilds involved in chain elongation: sugar-elongating organisms (SEOs), sugar fermenting organisms (SFOs), hydrogenic sugar fermenters (HSFs), lactate-elongating organisms (LEOs), ethanol-elongating organisms (EEOs), and homoacetogenic organisms (HAOs).
- We simulated a well-studied chain elongating bioreactor to test the predictive power of the model and to diagnose bottlenecks to medium-chain fatty acid (MCFA) production.
- Simulation results suggest that MCFAs are produced by a single guild in the bioreactor by converting sugars to MCFAs.
- Lactate is predicted to be produced as an intermediate but only used to produce butyrate rather than MCFAs.

BACKGROUND: Chain elongation has been proposed as a process to recover valuable products from complex organic waste streams[3]. Of particular interest is the production of medium-chain fatty acids (MCFAs) containing 6 to 12 carbon atoms. The targeted production of MCFAs over short-chain products remains a challenge. While strategies to increase MCFA specificity have been explored, including modifying ratios of electron donor to electron acceptor and real-time extraction of products[73, 74], we used metabolic models to investigate potential physiological drivers for MCFA production. From simulation results, we hypothesize several strategies to increase MCFA production.

RESULTS & DISCUSSION: Two metabolic models were constructed, expanding the range of substrates, products, and biochemical pathways of existing mixed-culture fermentation models[75-77]. First, a single cell metabolic model (iFermCell215) representing the combined metabolic capabilities of all functional guilds within a single unit was used to investigate substrates that may favour MCFA production. iFermCell215 allows for the free exchange of metabolic intermediates and cofactors between pathways and represents an organisms that can perform the metabolic activities of all the functional guilds. It can be used to identify sets of reactions that may favor MCFA production. Modelling results suggested that ethanol, but not lactate, increased MCFA production. Modelling results also suggest that an ideal ratio of acetate to ethanol is 0.56 mol acetate per mol ethanol which aligns with recent observations by others[73]. Second, a guild-based metabolic model (iFermGuilds789) was used to simulate the behaviour of a well-studied bioreactor that contained four functional guilds[18, 78]. The guilds were constrained according to their relative abundance in the bioreactor. When constraining this model to produce the products observed in the bioreactor, it was predicted that all of the MCFAs were produced by sugar-elongating organisms. While lactate-elongating organisms were present in the reactor, they were predicted to convert lactate into butyrate as a sole product. Third, we used the models to explore specific metabolic features and their impacts on MCFA production. We found that while energy conserving mechanisms can improve growth, they can be detrimental to MCFA production. For instance, the models predicted that energy conserving hydrogenases (ECH, HydABC) decreased MCFA production compared to the use of non-energy conserving hydrogenase. In these cases, the presence of alternative energy-conserving mechanisms obviate the need to conserve energy via reverse b-oxidation coupled to proton translocation with the RNF complex. These results demonstrate that metabolic features outside of reverse b-oxidation impact the primary fermentation products.

CONCLUSION: This study demonstrates the value of metabolic modelling to augment other omics techniques to assess chain elongation bioreactors. Modelling results suggest that in carbohydrate-based platforms, conversion of sugars to lactate may be undesirable. Instead, organisms that directly convert sugars to MCFAs may be desirable. Further, the model results highlight differences between ethanol and lactate as intermediates. While ethanol is

predicted to increase MCFA production, lactate may not. In addition to the modelling results, we also propose a guild-based framework for analysing and assessing chain elongation bioreactors and the models described in this work are available to the chain elongation research community (https://github.com/mscarbor/Mixed-Culture-Fermentation-Models).

Poster presentation: Co-culture of Lactobacillus and Megasphaera species to produce caproic acid by mimetic microbiome system using food wastes

Authors

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Mr. Sung Min Han - Hanyang university

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Dr. Okkyoung Choi - Hanyang university

Prof. Byoung-In Sang - Hanyang university

Abstract

BACKGROUND: Many chain elongation processes have used microbiome to produce medium chain carboxylic acids (MCCAs) from biowastes. *Megasphaera* species, purely isolated strains, are representative bacteria known to produce caproic acid using various substrates. However, they were rarely found in environmental process for MCCAs production. The production of caproic acid was observed by mimetic microbiome system by coculture of *Megasphaera* and *Lactobacillus*. *Lactobacillus* species can convert complex carbohydrates such as starch in food wastes to lactate, used as a substrate by *Megasphaera* species for MCCAs production.

RESULTS & DISCUSSION: As a result of coculture of *Lactobacillus amylovorus* and *Megasphaera hexanoica*, 12 g L^{-1} of caproic acid was obtained from food wastes in a batch culture. In a reactor with *in-situ* biphasic extraction system with supplied food wastes, caproic acid production was maintained stably.

CONCLUSION: By mimetic microbiome, caproate was stably produced using food waste. Using the purely isolated strains was excellent in securing the stability of the reactor because enrichment of each strain was possible.

Poster presentation: Caproic acid production from lactate using Megasphaera hexanoica

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Abstract

HIGHLIGHTS:

- *Megasphaera hexanoica* can metabolize lactate to produce caproic acid.
- High yield of caproic acid was achieved.
- Caproic acid production per unit cell concentration under lactate condition was higher than that of fructose condition.

BACKGROUND: Lactate can be readily produced from carbohydrate-rich wastes such as food waste by environmental microorganisms and used as an electron donor to produce various medium chain carboxylic acids(MCCAs) through chain elongation process [1]. Although MCCAs production from various wastes using microbial communities has been conducted [2,3], there have been few reports of isolated strains capable of producing MCCAs from lactate. In this study, we showed *Megasphaera hexanoica*, a bacterial strain known to produce MCCAs from fructose, can metabolize lactate to produce caproic acid. The yield and rate of caproic acid production using lactate were also investigated and compared with the results using fructose.

RESULTS & DISCUSSION: Using lactate as an electron donor, caproic acid production was observed in acetate and butyrate containing medium. Although

the cell growth of *M. hexanoica* using lactate was much lower than using fructose, the titre of caproic acid showed no significant difference. Under lactate condition 9.3 g/L of caproic acid was produced and 0.3 g/L/h of caproic acid production rate was achieved. In addition, when using lactate, the amount of caproic acid per unit cell concentration was over 2 times higher than using fructose.

CONCLUSION: *M. hexanoica* metabolized lactate to produce caproic acid and achieved high titre of 9.3 g/L. Increasing the cell concentration of *M. hexanoica* in the condition of using lactate is expected to improve caproic acid productivity. Since this strain appears to have high MCCAs production capacity under lactate conditions, it can be applied to produce MCCAs in the lactate fermentation with various wastes.

Bioreactor engineering and bioprocess development

Invited presentation: Chain elongation – friends and foes

DOI: https://doi.org/10.18174/icec2020.18025
Prof. Ramon Ganigue - Center for Microbial Ecology and Technology; Ghent University, Belgium

Understanding oscillation in gas fermentation

DOI: https://doi.org/10.18174/icec2020.18026

Authors

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Abstract

Gas fermentation can play a crucial role in the circular economy. However, oscillations have been observed in gas fermentation. Oscillatory behaviour provides a unique opportunity for understanding the robustness of metabolism, as cells respond to changes by inherently compromising metabolic efficiency. Here, we quantify the limits of metabolic robustness in self-oscillating autotrophic continuous cultures of the gas-fermenting acetogen *Clostridium autoethanogenum*. On-line gas analysis and high-resolution temporal metabolomics showed oscillations in gas uptake rates and extracellular by-products synchronised with biomass levels. We found that the intrinsic nature of gas fermentation allows for an initial growth phase on CO, followed by growth on CO and H_2, after which a down cycle is observed in synchrony with a loss in H_2 uptake. Intriguingly, oscillations are not linked to transnational control as no differences were observed in protein expression during oscillations. Intracellular metabolomics analysis revealed decreasing levels of redox ratios in synchrony with the cycles. We used a thermodynamic metabolic flux analysis (tMFA) model

to investigate if regulation in acetogens is controlled at the thermodynamic level. By incorporating endo and exo-metabolomics data into the model we could show that the thermodynamic driving force of critical reactions collapsed as H_2 uptake is lost. The oscillations are coordinated with redox. The data indicate that metabolic oscillations in gas fermentation acetogens are controlled at the thermodynamic level. The work presented will show that thermodynamic control of metabolism, potentially contributing to metabolic efficiency in gas fermentation.

Microbial recycling of biodegradable plastic PLA (poly lactic acid) into a spectrum of bioplastic precursors by anaerobic fermentation

Authors

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The effect of nano zero-valent iron on chain elongation

DOI: https://doi.org/10.18174/icec2020.18027

Authors

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Abstract

Nano zero-valent iron (NZVI) enhance anaerobic digestion by acting as electron donor or pH buffer. Currently, dose NZVI promote CE is not clear. The effect of NZVI on CE was demonstrated in this research. Caproate production was enhanced with NZVI addition. 5 g/L NZVI can improve caproate production by 100%. NZVI can help improve ethanol oxidation and reduce intermediates accumulation. Also, NZVI can improve H_2 production and prevent pH to decrease. *Oscillibacter Marseille-P3260 and Corynebacterium* was found promoted by NZVI. Besides, *Caloramator* was also significantly enriched with NZVI addition, which suggest a potential electron path between NZVI corrosion and chain elongation.

Impact of substrate concentration on granular fermentation for caproic acid production

DOI: https://doi.org/10.18174/icec2020.18028

Authors

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Abstract

HIGHLIGHTS:

- Lower substrate concentrations do not affect caproic acid selectivity.
- Higher substrate concentrations give rise to toxic caproic acid concentrations, which shifts production towards butyric acid.
- Lowering substrate concentrations induces biomass aggregation but the mechanism remains unclear.

BACKGROUND: Todays increasing organic waste production poses a challenge for the conversion of liquid waste streams into added-value products beyond non-profitable methane and ethanol-based medium chain carboxylic acids (MCCA). Over the past decade, lactic acid chain elongation to produce caproic acid (C6) has gathered an increasing amount of attention due to its potential in coupling lactic acid production from carbohydrates to chain elongation, thereby eliminating the need for exogeneous addition of electron donors[22, 79]. To date

these systems, however, still struggle with rate limitations, a key barrier that had been identified early on in the new wave of MCCA-research of the 2010s[80]. Carvajal-Arroyo et al. (2019)[11] recently demonstrated the possibility of C6 production via chain elongation using granular reactor technology fed with thin stillage, a biorefinery side-stream containing approximately 20 $g \cdot L^{-1}$ of carbohydrates, without external addition of electron donors[11]. By retaining high biomass concentrations as granular biofilm, that study enabled an order of magnitude increase in C6-production rates, up to 13.7 $gC6 \cdot L^{-1} \cdot d^{-1}$, compared to a suspended biomass system on a similar stream[25]. Understanding the effects of the feedstock composition, concentration and load on the chain elongation and biomass aggregation in this novel system form the crucial next steps towards feedstock diversification. The aim of this study was to shed light on the effect of substrate concentration, with solids-free thin stillage as feedstock, on granular fermentation in expanded granular sludge bed (EGSB) reactors.

RESULTS & DISCUSSION: In a first part, diluting the thin stillage (44.15 ± 2.64 $gCOD \cdot L^{-1}$) to respectively 75%, 50% and 25% of the original COD content resulted in a decrease in C6 concentration (from 4.35 ± 0.25 $g \cdot L^{-1}$ at 100% to 1.59 ± 0.27 $g \cdot L^{-1}$ at 25%) in the effluent while the selectivity with which C6 was produced, remained constant at 48 ± 3% (COD of C6 relative to all produced carboxylic acids) (Figure 21). Additionally, lower substrate concentrations resulted in higher substrate conversion efficiencies (Figure 21). Furthermore, the highest amounts of total biomass (sum of planktonic and granular) were consistently found at the lowest substrate concentrations and substantial growth of the granular bed was observed at lower substrate concentrations. This suggests that substrate limitation and/or concomitant low C6 concentrations may have a positive impact on biomass aggregation, but the mechanism remains unclear.

In a second part, amending the solids-free thin stillage with D-glucose to achieve 110%, 125% and 200% of the original COD content did not result in a proportional increase in C6 concentration (Figure 21). Instead, butyric acid started accumulating up to similar concentrations (4.03 ± 0.48 $g \cdot L^{-1}$ at 125% compared to 1.78 ± 0.20 $g \cdot L^{-1}$ at 100%) as C6 (4.35 ± 0.49 $g \cdot L^{-1}$). We hypothesized that this was due to product toxicity exerted by C6. This hypothesis was supported by batch experiments, which yielded similar results when feeding three times the carbohydrate concentration. Additionally, lower substrate

conversion rates were obtained at higher initial C6 concentrations. In terms of granular biomass behaviour, higher substrate concentrations resulted in visually larger granules.

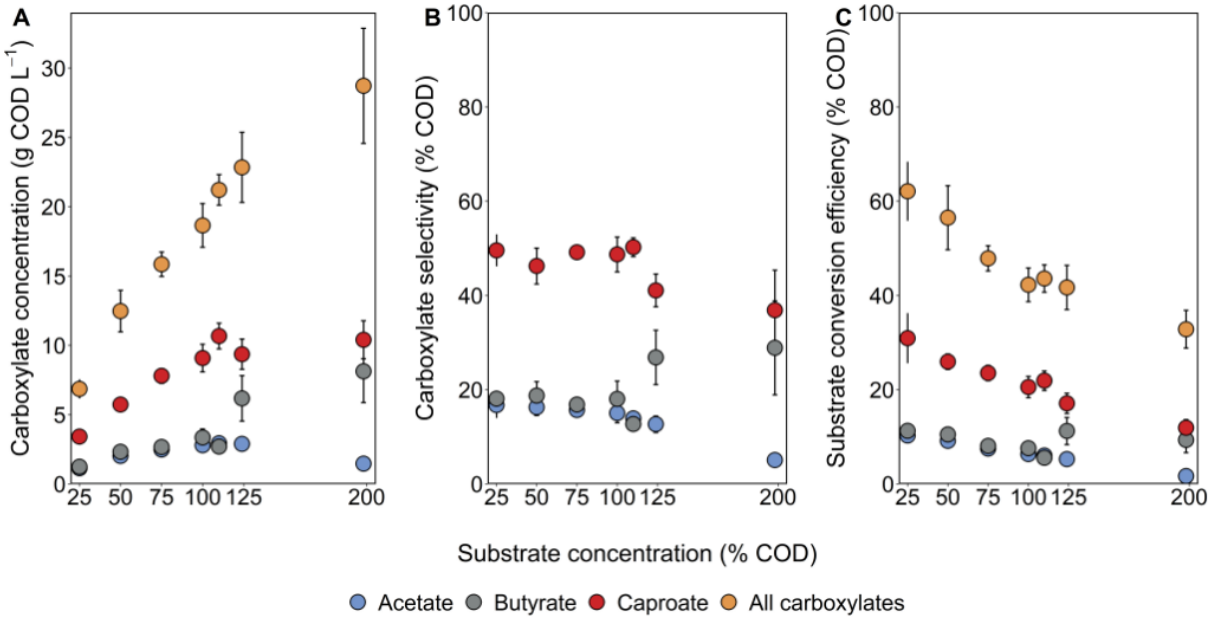

Figure 21. Carboxylic acid concentration (A), selectivity (B) and substrate conversion efficiency (C) in function of the substrate concentration.

CONCLUSION:

The constant selectivity and increased substrate conversion efficiencies at low substrate concentrations demonstrate that high COD concentrations are no prerequisite for caproic acid production in an EGSB. However, the low C6 concentrations may pose challenges for efficient product extraction. At higher substrate concentrations, selectivity for caproic acid decreases due to product toxicity, implicating in-situ product extraction is necessary to maintain high selectivities. Overall, this study demonstrates that chain elongation in expanded granular sludge beds offers untapped potential and could be expanded towards more dilute and concentrated waste streams.

Food waste served three ways: butyric, lactic or caproic acid

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Abstract

HIGHLIGHTS:

• Operating strategy influenced microbial community development, and the predominant metabolic product in food waste fermentation
• Chain elongation was stimulated at higher retention times
• Lactic acid was the dominant product at higher organic loads

BACKGROUND:

Effective food waste (FW) management must provide cost-efficient resource and energy recovery to enable a sustainable circular bio-economy[81]. Of particular interest is fermentation with anaerobic mixed microbial cultures (MMC) to generate products, such as biogas, lactic acid, ethanol, H2, or carboxylic acids (CA) ranging from volatile fatty acids (VFA), to medium chain carboxylic acids (6-8 carbon atoms, MCCA) via chain elongation. Various factors dictate fermentation outcome and MMC composition, e.g. the organic loading rate (OLR) and retention times[82, 83]. To improve chain elongation yields in FW fermentation, various reactor configurations, e.g. two-stage or leach bed, and supplementation of chemicals have been considered[84, 85]. However, studies have shown MCCA production from FW is possible in a single-stage stirred tank reactor (STR)[33]. The current study aimed to improve our understanding of how operation of a single-stage STR steers product outcome and the microbial community. The effects of OLR and hydraulic retention time (HRT) were assessed separately by operating three sets of duplicate semi-continuous STR over three HRT. We compared operation at a baseline HRT of 8.5 days and OLR of 12 gCOD L -1 d -1 (LH/LO) to operation at a higher HRT of 10.5 days (HH/LO) and a higher OLR of 20 gCOD L -1 d -1 (LH/HO). FW was collected from a

full-scale industrial anaerobic digestion (AD) plant (GENeco, UK) and seeding cultures from in-house acidogenic fermentation reactors.

RESULTS & DISCUSSION:

In previous experiments, we found that the collected FW from the AD plant varies slightly in composition, is high in biodegradable total COD (130 to 163 gCOD L-1) and already contains ethanol (5 to 13 g L-1) and lactic acid (around 20 g L-1). The three sets of operating conditions resulted in three different fermentation outcomes and MMC enrichments (Figure 22).

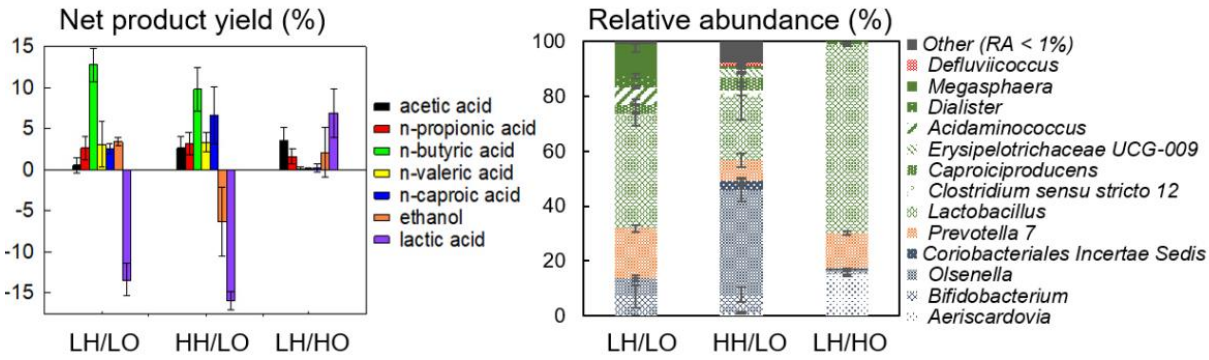

Figure 22. Average net yields of liquid fermentation outputs (left) and MMC 45 composition at the genus level (right, colour per phylum) for varying operating conditions (i.e. combinations of HRT and OLR).

At lower OLR, lactic acid was net consumed and the total CA yields were similar, yet product distribution differed. In the LH/LO reactors, 55 ± 14% of the CA was butyric acid, and the fermentation was enriched in lactic acid bacteria and VFA-producers typical of primary acidogenic fermentation. At longer HRT in the HH/LO system, over three times more caproic acid was obtained (up to 6.2 g L -1). The semi-continuous fermentation cycle showed consecutive fermentation stages of acidogenic fermentation and chain elongation in HH/LO, in line with earlier reports on chain elongation in FW6 . The net consumption of both ethanol and lactic acid, and the presence of genera related to ethanol- and lactate-based chain elongation, e.g. Caproiciproducens, Clostridium sensu stricto 12 and Olsenella spp., indicate chain elongation could occur with either or both electron donor. Thus, operating at longer HRT stimulated chain elongation. The residual butyric acid and ethanol concentrations in the effluent of HH/LO suggest the process could be further optimised. When operating at higher OLR (LH/HO) minimal CAs were detected and predominantly lactic acid accumulated (32 ± 5 gCOD L-1). More than twice the amount of NaOH was required to correct pH compared to operations at lower OLR. This was reflected in a MMC enriched in lactic acid producing and acid-resistant Lactobacillus and Aeriscardovia spp.

CONCLUSION:

Food waste, with high biodegradable COD content, can serve as a feedstock for a range of platform chemicals. This can be achieved in a simple STR setup by manipulation of the operating conditions. Operating at a high OLR resulted in an organic overload, characterised by acidogenic lactic acid accumulation, where an acid-resistant community thrived. At lower OLR, an increased residence time allowed the community to evolve to perform chain elongation via secondary fermentation after acidogenesis. These results improve our understanding of the underlying metabolic pathways in food waste fermentation, and demonstrate the potential of adapting current single-stage food waste AD systems to produce other bio-chemicals by modifying HRT and OLR.

Effects of zero-valent iron nanoparticles on lactate-based chain elongation

Authors

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Abstract

HIGHLIGHTS:

- nZVI affected chemical and biological processes.
- n-caproate was formed only in the presence of nZVI.
- nZVI could potentially be replaced with (bio)electrochemistry.

BACKGROUND: Production of biochemicals from renewables is of outmost importance to reduce anthropogenic impact on the environment. A mixture of carboxylates (acetate [C2], propionate [C3], n-butyrate [nC4], n-valerate [nC5], n-caproate [nC6]) can be produced through chain elongation of substrates such as lactate and steering to MCC is desired since these are easily separated and used in the chemical and food industries[3]. Chain elongation can be steered with different operational conditions such as pH, HRT, electron donor-to-acceptor ratio and hydrogen partial pressure[3]. In this study, zero-valent iron nanoparticles (nZVI) were tested as catalyst to steer product formation in chain elongation. Dosing nZVI could stimulate several enzymes involved in lactate metabolism such as lactate enantiomers interconversion, lactate oxidation, pyruvate oxidative decarboxylation, hydrogen formation and energy conservation. Carbon dioxide (CO_2) recovery via acetate or alcohol formation could also be promoted. These effects would be reflected in altered conversion rates and product spectra.

RESULTS & DISCUSSION: Addition of nZVI to a mixture of lactate monomers and oligomers (polyesters) resulted in chemical hydrolysis of lactate oligomers by

alkaline de-esterification (Figure 23). Under fermentative conditions, lactate and acetate were elongated to nC4-nC6 carboxylates with hydrogen release to the headspace. This chain elongation phase was followed by a hydrogen/CO_2 consuming phase for acetate formation. Lactate conversion rates were improved at ≤2 g nZVI·L^{-1} promoting n-caproate production. n-caproate was not produced in the control experiment without nZVI but reached 4.3±0.3 g·L^{-1} at 1 g nZVI·L^{-1}. Propionate formation became relevant when ≥3.5 g nZVI·L^{-1} were added. CO_2 recovery was not clearly increased with nZVI (Figure 24). Both lactate enantiomers (D-lactate and L-lactate) were racemized during chain elongation and converted into even-chain carboxylates at different rates.

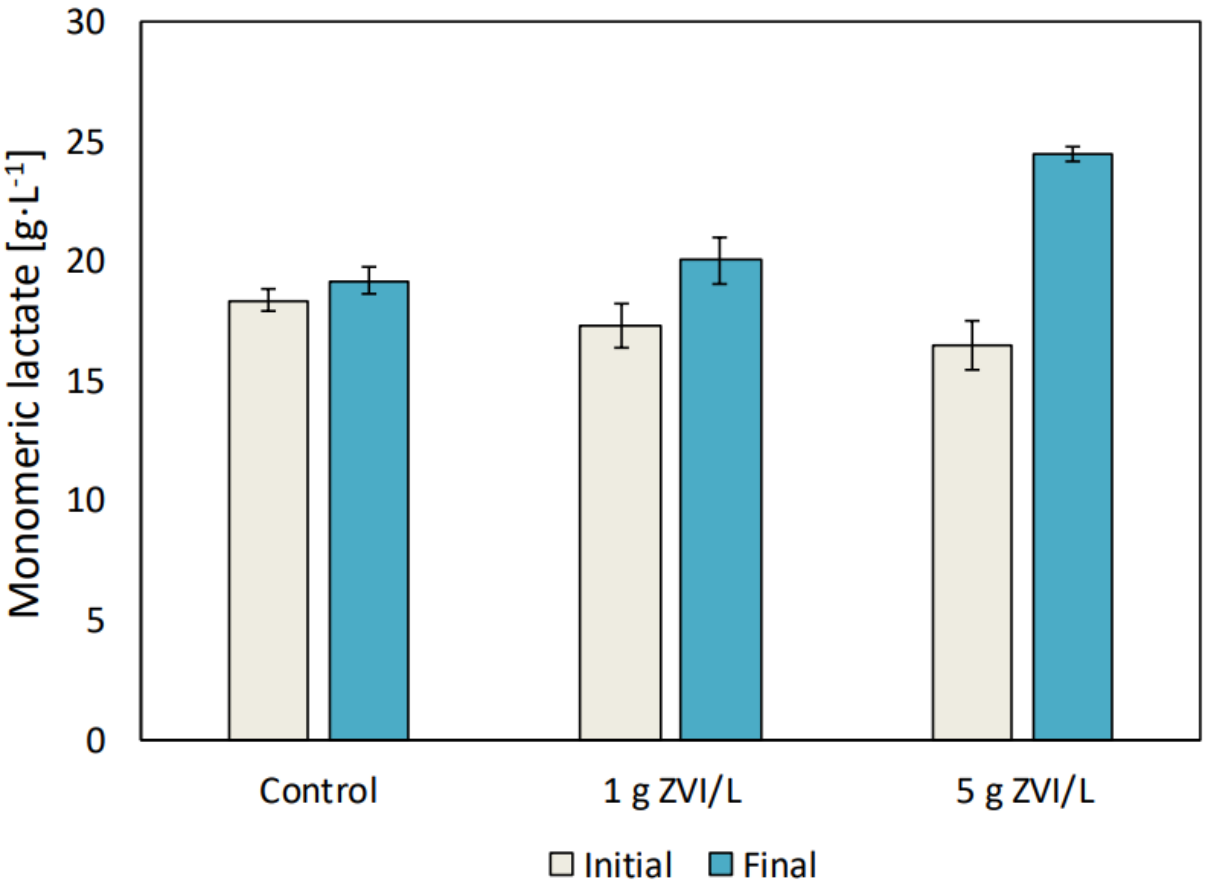

Figure 23. Increase in lactate monomers due to lactate oligomers hydrolysis.

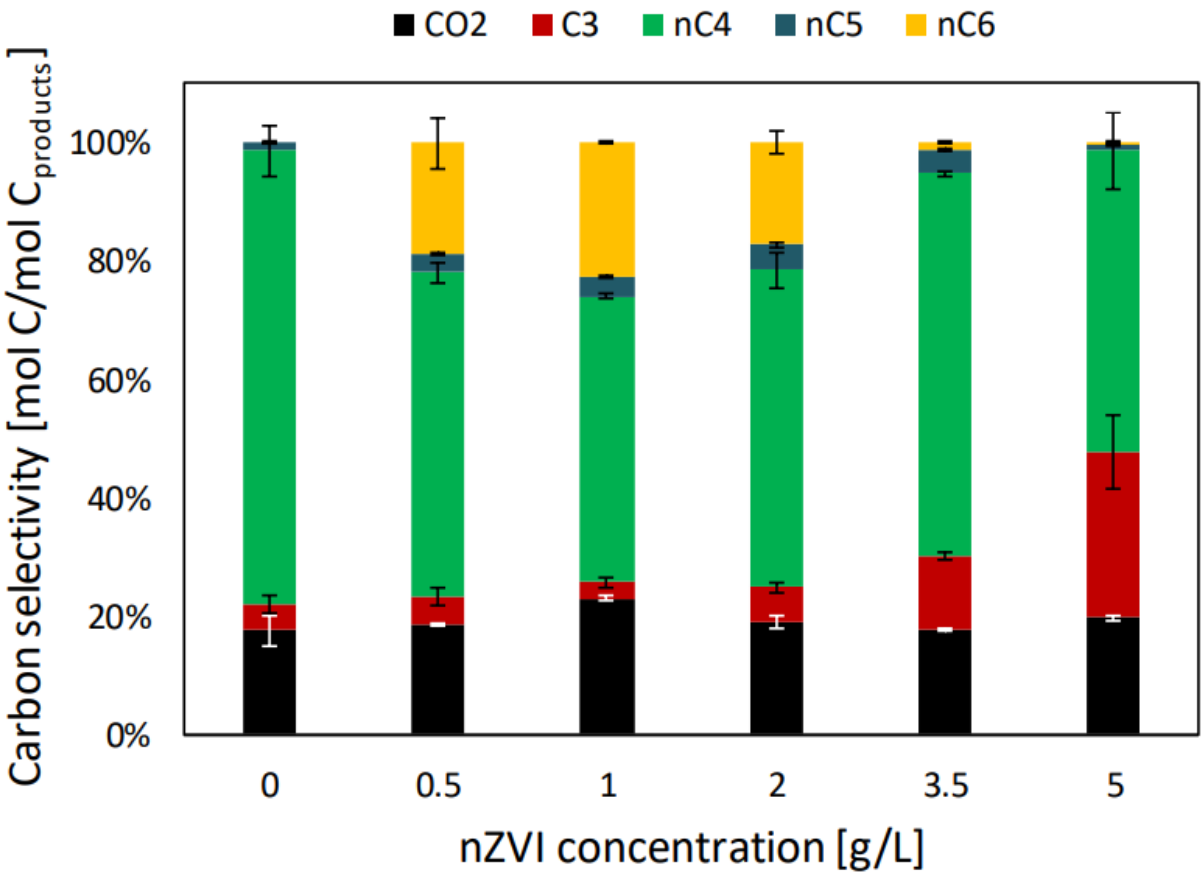

Figure 24. Effect of nZVI concentration on product spectrum.

CONCLUSIONS: nZVI enhanced hydrolysis of lactate oligomers increasing substrate availability. Lactate-based chain elongation products and rates were affected in a dose-dependent manner by nZVI with no observed microbial activity inhibition. The effects of nZVI could be on the oxidation-reduction conditions, electron donor and pH changes. Lactate enantiomeric proportions were observed to be determined by the type of metabolism (chain elongation vs acrylate pathway) and, therefore, feeding D-lactate in continuous reactors would not necessarily translate into higher chain elongation rates. The different effects of nZVI may be improved in different system configurations and partially replaced with (bio)electrochemical systems.

Invited presentation: Microbial electrosynthesis - a techno-economic driven roadmap towards implementation

DOI: https://doi.org/10.18174/icec2020.18030

Prof. Ludovic Jourdin – TU Delft

ABSTRACT: Microbial electrosynthesis (MES) allows carbon-waste and renewable electricity valorization into industriallyrelevant chemicals. MES has received much attention in laboratory-scale research, although a technoeconomic-driven roadmap towards validation and large-scale demonstration of the technology is lacking. In this work, two main integrated systems were modelled, centered on (1) MES-from-CO2 and (2) MES from shortchain carboxylates, both for the production of pure, or mixture of, acetate, n–butyrate, and n–caproate. Twenty eight key parameters were identified, and their impact on techno-economic feasibility of the systems assessed. The main capital and operating costs were found to be the anode material cost (59%) and the electricity consumption (up to 69%), respectively. Under current state-of-the-art MES performance and economic conditions, these systems were found non-viable. However, it was demonstrated that sole improvement of MES performance, independent of improvement of non-technological parameters, would result in profitability. In otherwise state-ofthe-art conditions, an improved electron selectivity (≥36%) towards n-caproate, especially at the expense of acetate, was showed to result in positive net present values (i.e. profitability; NPV). Cell voltage, faradaic efficiency, and current density also have significant impact on both the capital and operating costs. Variation in electricity cost on overall process feasibility was also investigated, with a cost lower than 0.045 € kWh−1 resulting in positive NPV of the state-of-the-art scenario. Maximum purification costs were also determined to assess the integration of a product's separation unit, which was showed possible at positive NPV. Finally, we briefly discuss CO2 electroreduction versus MES, and their potential market complementarities.

Boosting a biocathode by analysis: the invasive effects of cyclic voltammetry on bioelectrochemical chain elongation

Authors

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Prof. Cees Buisman - Wageningen University

Prof. Harry Bitter - Wageningen University and Research

Abstract

HIGHLIGHTS:

- Cyclic Voltammatry use for analysis during microbial electrosynthesis disturbed the system operation and resulted in a current increase lasting up to three weeks
- Cyclic Voltammetry resulted in the release of biomass and metal compounds from the biocathode

BACKGROUND: Bioelectrochemical chain elongation is a promising method for the production of valuable chemicals from waste streams, such as CO_2. A microbial electrosynthesis system generally exists of an anode where an oxidation reaction produces electrons, which are supplied to a cathode where a biofilm grows. The bacteria in the biofilm use the electrons for chain elongation conversions. By using an electrode for the electron supply, renewable electricity could be used to upgrade CO_2 to longer chain carboxylates such as acetate, butyrate and caproate. Although offering nice perspectives, the technique still requires a greater understanding of its mechanisms and steering tools. From the field of electrochemistry, various techniques are available for the analysis and characterization of conversions and electrode composition. One such technique is Cyclic Voltammetry (CV). During a CV scan, the potential is subsequently increased and decreased while measuring the resulting current in the system. This potential scan is widely used in bioelectrochemistry to show biological activity. A disadvantage from the technique is that the potential change rate

during the scan has to be kept very low to avoid background signals. Using this low scan rate allows for changes at the cathode that could be irreversible. The aim of our study was to investigate these changes and their effect on the performance of a bioelectrochemical chain elongation reactor.

RESULTS & DISCUSSION: After applying CV on a biocathode in which CO_2 was continuously elongated to acetate at a constant current, the cathodic current increased. This increase lasted for four days up to several weeks. To further elucidate the mechanisms behind this increase, the metal composition and suspended cell concentration of the catholyte were investigated at different moments during and after the scan. The concentrations of Fe, Co, Al, Ba, Mn, Mo and biomass increased in the biotic catholyte predominantly during the oxidation peak of the first CV cycle, indicating these compounds were released from the cathode (Figure 25). The catholyte metal concentrations decreased rapidly within four hours after the CV at the cathodic operation potential of -0.85 V vs SHE suggesting they are re-deposited.

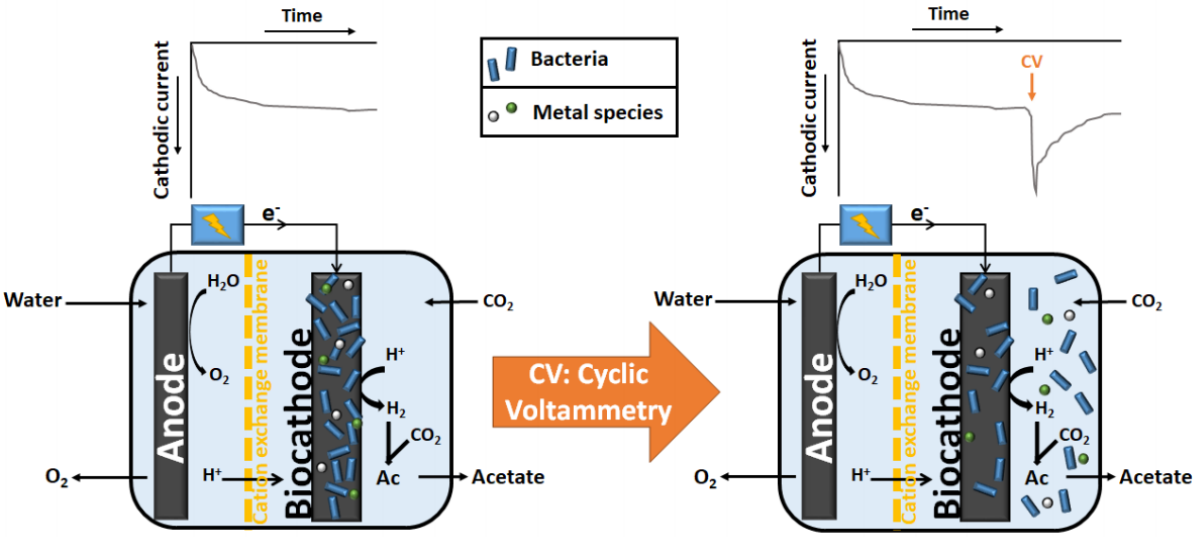

Figure 25. Performing Cyclic Voltammetry on a bioelectrochemical chain elongation system caused a current increase and a release of metal compounds and biomass from the cathode.

CONCLUSION: Our study showed that CV is not an innocent analysis technique, but causes irreversible changes at the cathode. These changes induced a system boost in the form of an increased electron supply to the cathode. Our findings form a starting point for follow up studies investigating the mechanisms behind the catalytic effects of the performed analysis method.

Bio-electroCO2recycling to C4-C6 products in two steps

DOI: https://doi.org/10.18174/icec2020.18031

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Abstract

BACKGROUND:

Microbial Electrochemical Technologies (METs) is an emerging technology field where SCFAs are produced from CO_2 and electricity[86, 87]. Recent studies establish ethanol is the most added-value product that can economically and sustainably be produced through METs[88]. Despite SCFA are valuable themselves, they can be elongated *via* chain elongation, to more valuable products as MCFAs[89]. MCFAs are preferable due to their higher combustion energy and broad industrial applications as animal feeds or pharmaceuticals[3]. However, they are barely produced through METs excepts few examples at long-term operation[90, 91]. In this study, a two-step system for the conversion of CO_2 into C4-C6 commodity chemicals is proposed (Figure 26). The first step consisted in the bio-electroCO_2 recycling into acetate and ethanol. These products were employed as substrates to perform chain elongation process to commodity chemicals (C4 and C6 compounds).

RESULTS & DISCUSSION:

MET platform aimed at reaching the broth requirements for the following up process (fermenter). The cathode potential of several bioelectrochemical systems (BES) were poised at -0.8 V *vs.* SHE to ensure H_2-bio-hydrogen-mediated production of commodity chemicals from carbon dioxide[92]. Initially optical density (OD) was poor and a brief adaptation period was needed, then, after 30 days of operation productions of acetate and ethanol were achieved and 1:1 ratio was achieved[93].

The second step consisted in several 120mL batch fermenters in triplicate (Table 2). High hydrogen partial pressure (pH_2) in the reactor was required for the growth of the cultures and perform chain elongation. Hydrogen produced in first step can be recycled. pH was controlled and restored if necessary, in each fermenter. Both tests were performed in batch, incubated at 25 ± 1 ºC and kept in the dark.

Table 2. Tests performed in the second step of the process.

Test	CO2:H2	Ethanol/Acetate ratio	pH
1	20:80	1:1	7
2	20:80	1:1	5.5

In test 1, it took a short adaptation period, 15 days until chain elongation started and less than 20 days to get C6 production. Butyrate and caproate concentrations were 33.88 ± 4.02 and 44.99 ± 0.41 mM C and production rates were 7.88 and 11.76 mM C d^{-1}, respectively (Figure 26). While in test 2 both, activity and productions were much lower (Figure 26).

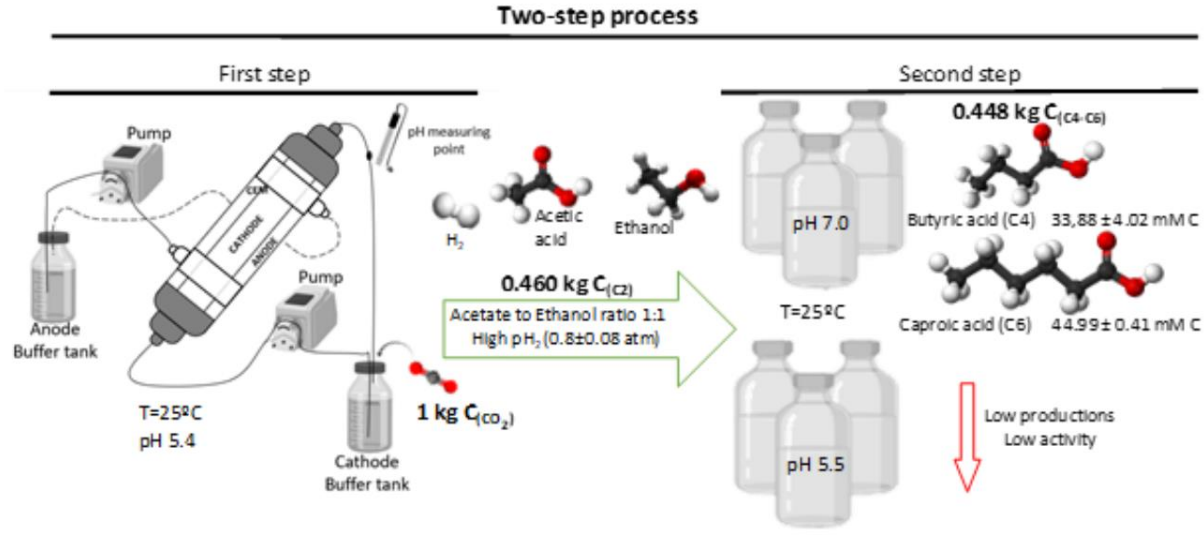

Figure 26. Schematic representation of the bio-electroCO2recycling platform. CEM: Cation Exchange Membrane.

CONCLUSION:

This study presents a bio-electroCO_2recycling platform to produce butyrate and caproate from carbon dioxide. Optimal conditions were high pH_2, pH around 7 and the more ethanol availability, the more elongation and product selectivity. First step is the limiting one due to its low carbon use, compared to the second one. Although results are promising, further research is needed since it requires greater production rates and more valuable products (e.g. octanoic acid).

The role of inorganic carbon in a hydrogen-based Membrane Biofilm Reactor

Authors

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Abstract

Biological conversion of CO_2 into biofuels and/or industrial feedstock such as carboxylates is an excellent carbon-cycling strategy. The use of autotrophic anaerobic bacteria in the membrane biofilm reactor (MBfR) is a promising an effective system to convert the electron equivalents in hydrogen gas to acids and alcohols. We operated a hydrogen-based MBfR for production of short-chain carboxylates (SCCs) and medium-chain carboxylates (MCCs). We evaluated the impacts of hydrogen flux, inorganic carbon (IC) concentration, and hydraulic retention time, on acetogenesis and microbial chain elongation (MCE). We achieved acetogenesis for all tested conditions in the MBfR, being the highest acetate concentration 55 mM. The availability of the carbon source was the key factor for MCE of SCCs to MCCs. When IC was limited, chain elongation occurred up to caproate (C6). Conversely, acetate was the only product when IC was amply available, with hydrogen as the limiting factor. The proper management of carbon availability and hydrogen supply in the MBfR allows control over carbon chain length of the carboxylates produced.

Harnessing Hydrogen Production during Microbial Chain Elongation for Reduction of Oxidized Groundwater Contaminants

Authors

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Ms. Sayalee Joshi - Arizona State University

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Dr. Anca Delgado - Arizona State University

Abstract

BACKGROUND: H_2 is a universal electron donor for reduction of many oxidized contaminants including the chlorinated solvent, trichloroethene (TCE). Reductive dechlorination of TCE using the organohalide respiring bacteria *Dehalococcoides mccartyi* is the most common bioremediation approach intrinsically dependent on H_2. *Dehalococcoides mccartyi* use H_2 as sole electron donor to reduce TCE to ethene through the intermediates dichloroethene and vinyl chloride. During bioremediation or in the laboratory for growth of *Dehalococcoides mccartyi* mixed cultures, H_2 is typically supplied through fermentation of substates such as, lactate, glucose, and emulsified vegetable oil. However, some fermentation pathways are H_2 deficient, such as lactate to propionate and glucose to ethanol. One reliable H_2-producing microbial process is chain elongation of carboxylates. Although, the ability of chain elongation to produce H_2 has been known for more than eight decades, its application in bioremediation has not been studied. The objective of this work was to explore microbial chain elongation as a novel approach and alternative to typical fermentation for generating the required H_2 for reductive dechlorination of TCE by *Dehalococcoides mccartyi* in batch experiments.

RESULTS & DISCUSSION: We established soil microcosms with TCE, 50 mM acetate and 50 mM ethanol as chain elongating substrates, 10 g of soil and with laboratory medium or natural groundwater from a TCE-contaminated site in

Arizona, USA. The microcosms were inoculated with the TCE bioaugmentation cultures ZARA-10 and SDC-9 containing *Dehalococcoides mccartyi* and with a mixed-community enrichment developed from soil containing the chain-elongating microorganism *Clostridium kluyveri*, among other members of *Firmicutes*. Transfer subcultures were created from these microcosms and were fed with TCE and 50 mM acetate and 50 mM ethanol, or 100 mM ethanol.

In the inoculated soil microcosms, acetate and ethanol were elongated to butyrate, butanol, and caproate. Up to 6 mmol L^{-1} H_2 (nominal concentration) accumulated in the microcosms by day 7. Reductive dechlorination of the added TCE occurred simultaneously with chain elongation. Approximately 2 mmol L^{-1} TCE was dechlorinated to ethene over 115 days without additional supplementation of acetate and ethanol. Once H_2 became non-detectable in the microcosms (after 20-50 days), a decrease in the concentration of butyrate and an increase in the concentration of acetate indicated that the sustained TCE reductive dechlorination was proceeding with H_2 from butyrate fermentation. The microcosms with natural groundwater showed somewhat slower dechlorination rates than those with reduced anaerobic medium. This was likely due to the presence of other H_2-consuming electron acceptors from groundwater that were simultaneously reduced during incubation, including 8.6 mM sulfate, 0.3 mM nitrate, and 1.5 µM perchlorate. Regardless, even in the presence of additional electron accepting processes that potentially competed for H_2 with *Dehalococcoides mccartyi*, TCE dechlorination proceeded to mainly ethene.

Reductive dechlorination of TCE to ethene was also sustained in subsequent transfer subcultures with acetate and ethanol and with ethanol only as chain elongation substrates. In the transfers with ethanol only, the extent of chain elongation was enhanced and higher concentrations of caproate (up to 8.3 mM) were detected. Methanogenesis, a H_2-competing process for reductive dechlorination, was observed to a limited extent in the soil microcosms with TCE but was completely halted in the enrichment transfers. The H_2 dynamics observed may have given *Dehalococcoides mccartyi* an advantage over hydrogenotrophic methanogens resulting in complete reductive dechlorination of TCE.

CONCLUSION: Our data show that *Dehalococcoides mccartyi* can successfully use the H_2 produced from chain elongation to reduce TCE to ethene. This study provides fundamental knowledge positioning microbial chain elongation as a viable alternative to typical fermentation in bioremediation and possibly a more efficient approach for reliably producing H_2 for remediation of oxidized contaminants.

Modelling fermentative hydrogen production of cheese wastewater

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Abstract

HIGHLIGHTS:

- Bio-hydrogen recovery during secondary fermentation
- Lactate plays a fundamental role in carboxylic chain elongation
- Modified ADM1 simulated the syntrophism between species

BACKGROUND: Brazilian agroindustry has an expressive, but not exploited, source of energy in its wastewaters and by-products. For instance, cheese whey (CW) disposed from the dairy industry represents a high residual sugar-content (lactose) with potential to energy recovery by anaerobic bioprocess[94, 95]. In anaerobic microbiomes, sugar and other substrates are commonly hydrolyzed and fermented to short-chain mono-carboxylic acids (SCCAs), such as: acetic, propionic and butyric acids. In turn, the reverse β-oxidation pathway might transform SCCAs into medium-chain monocarboxylic acids, also known as chain elongation process (CE).

Despite organic acid outputs, both processes are well established as high biological hydrogen (bio-H_2) yielders. Also they are affected by carbon-source, key electron donors, partial hydrogen pressure (P_{H2}), pH, reactor microbiomes and temperature conditions[96, 97]. Additionally, the feasibility of electron donor source implies on CE-capable microorganisms activity, which can recover H_2

through SCCA consumption[3]. Conversely, P_{H2} can determine both fermentative and CE pathways, acting as thermodynamic inhibitor[98]. In addition, pH can also act as inhibitor in both processes, leading to electron control via disruption of bio-H_2 production pathways[99].

Mathematical modelling is a powerful tool to better understand complex interactions amongst microbiomes and inhibition factors, as presented so far. Thus, the aim of the present study is to develop a mathematical model to depict microbiome interaction between dark fermentative and CE biomasses. Experimental data to calibrate the model was gathered on a previous study of dark fermentative bio-H_2 production, in which batch essays of synthetic cheese whey wastewater were inoculated with continuous flow experiment biomass[100]. In order to consider chain elongation biomass (X_{CE}), kinetics and balances, along with P_{H2} and pH effects, a modified version of Anaerobic Digestion Model n.1 (ADM1)[101] is proposed.

RESULTS & DISCUSSION: The model was successfully implemented in MatLab®. In order to evaluate different microbial communities, biomass was split onto dark fermenters (X_{SU}) and X_{CE}. Latter was considered to grow during lactose fermentation and CE[102]. To avoid early CE synthesis (*i.e.* bio-H_2 and *n*-butyrate), X_{ce} was inhibited by a competitive lactate function. Which implied on four new parameters to be estimated (Table 3).

Table 3. Petersen matrix of proposed model, estimated parameters in bold.

Proc.↓ Comp.→	S_{su}	S_{bu}	S_{ac}	S_{lac}	S_{H_2}	X_{su}	X_{CE}	Rate
Uptake of sugar	-1		$f_{ac,su}$ $f_{bu,su}$	$f_{lac,su}$ $f_{lac,su}$	$f_{h2,su}$ $f_{bu,su}$	Y_{su}	Y_{CE}	$k_{m,su}\frac{S_{su}}{K_{s,su}+S_{su}}\mathbf{X_{su}}I_{pH}$ $\mathbf{k_{m,lac}}\frac{S_{su}}{\mathbf{K_{s,lac}}+S_{lac}}\mathbf{X_{CE}}I_{pH}$
Uptake of butyrate		-1						
Uptake of acetate			-1				Y_{CE}	
Uptake of lactate		$f_{bu,lac}$	$f_{ac,lac}$	-1	$f_{h2,lac}$			$\mathbf{k_{m,CE}}\frac{S_{lac}}{K_{s,CE}+S_{lac}}X_{CE}I_{pH}I_{H_2}I_{lac}$
Uptake of H_2					-1			
X_{su} decay						-1		$k_{dec}X_{su}$
X_{CE} decay							-1	$k_{dec}X_{CE}$

Due to reduced number of data between 75 to 150 h, a β-spline interpolation was used to better fit data. The initial X_{CE} / X_{SU} was kept constant on 0.167. Biomasses yields, sugar consumption kinetics and CE half saturation constants were based on literature[100, 103] and other parameters were adopted from ADM1

framework.

Time dependent simulation profiles indicated good agreement with data (Figure 27), with a slight early bio-H_2 production and diverging pattern on *n*-butyrate production around 150 h. Parameters were estimated by minimizing the sum of the absolute values of the deviations, presenting the following values: carbohydrates 0.88, acetate 0.16, *n*-butyrate 0.56, lactate 0.45, H_2 gas 0.04, biomass (not plotted) 0.27 and 0.16 for pH.

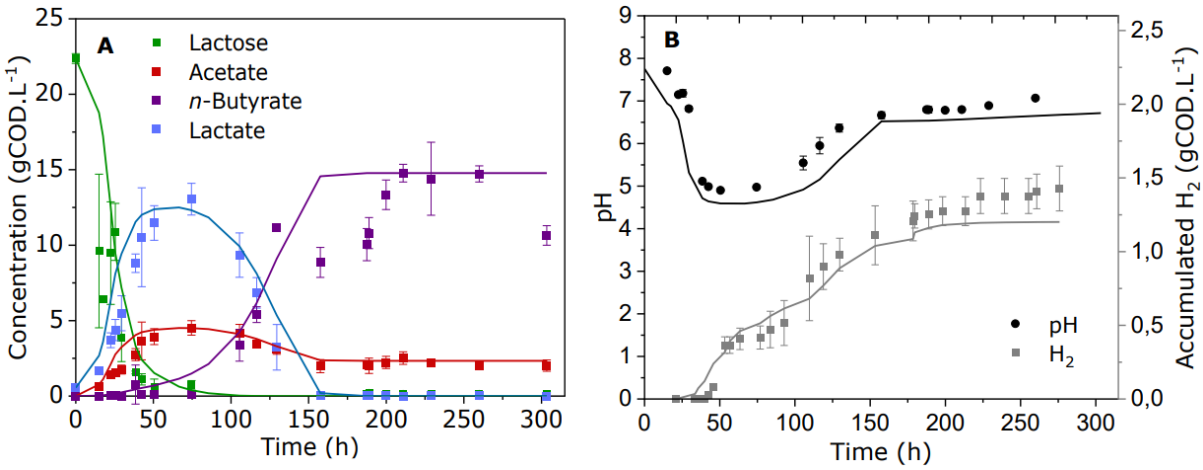

Figure 27. Experimental data (scatters) compared to simulation data (lines) obtained by modified ADM1 structure. A chart represents sugar and SCCAs dynamics, and B, accumulated H2 production and pH variation patterns.

CONCLUSION: The developed ADM1 model for acetate and lactate CE-coupled, considering syntrophism between two biomasses, could represent both bio-H_2 (%) and *n*-butyrate production.

Poster presentation: Parameters affecting chain elongation from syngas bioconversion

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Abstract

HIGHLIGHTS:

- Chain elongation in *C. kluyveri* is optimal at near neutral pH values
- Mixtures of acetic and butyric acids allow efficient hexanoic acid production
- Ethanol, acetic and/or butyric acids from syngas fermentation are suitable for chain elongation

BACKGROUND: The most common electron donor and electron acceptor for the production of hexanoic acid through chain elongation are ethanol and acetic acid, although they are not the only suitable ones. Acetic acid, and sometimes ethanol, can be obtained through different bioconversion processes such as the anaerobic digestion of solid waste[79], wastewater[104], or other feedstocks. The presence of other metabolites is not unusual, while some ethanol may need to be added if its concentration is limiting. Alternatively, the acetogenic bioconversion of syngas, as well as industrial emissions containing C_1 gases, such as CO and CO_2, will also yield acetic acid as an end metabolite. A limited number of anaerobic bacteria can also produce ethanol from C_1 gases, besides acetic acid[105]. Occasionally, it has been observed that butyric acid and even some hexanoic acid may also be obtained directly from C_1 gas fermentation by enriched anaerobic sludge[106] or by some pure acetogenic bacteria[107]. Optimizing aspects such as the pH of the medium[108], the composition of the fermentation broth or the nature and concentration of trace metals[108] allows to select for the preferred end metabolites. Volatile fatty acids (VFA) such as butyric acid may

thus be present, besides acetic acid, in such type of primary gas fermentation process, depending on aspects such as the nature of the biocatalyst, the pH, or the composition of the culture broth. Therefore, it is worth evaluating the effect of both acetic acid and butyric acid, individually or in mixture, as electron acceptors, as well as the effect of the composition of the culture medium and its pH, on chain elongation. Few recent studies have reported about the possibility to combine syngas fermentation with chain elongation[1], and it is thus also worth to study the effect of such parameters in integrated syngas fermentation and chain elongation processes. Therefore, the afore mentioned goals were the main objectives of the present research.

RESULTS & DISCUSSION: A first set of experiments was performed in automated suspended-growth bioreactors, under mesophilic conditions, with constant pH adjustment. With ethanol as electron donor, either acetic acid or butyric acid, individually, or their mixtures, all allowed the production of hexanoic acid through chain elongation with *Clostridium kluyveri*, using similar molar alcohol/acid ratios around 3.5 in all cases and initial ethanol concentrations around 15 g/L. However, the efficiencies in terms of growth rates and bioconversion were the highest with the mixture of acids and they were the lowest with pure butyric acid as single VFA. Typical growth rates of 0.039 h^{-1} were found with the mixture of VFA, while it dropped to 0.010 h^{-1} with butyric acid as single electron acceptor. There was no large difference between pure acetic acid (m_{max} = 0.031 h^{-1}) and the mixture of VFA though. On the other hand, increasing the initial available amount of electron donor (ethanol) to 25 g/L, while maintaining the same initial concentrations of VFA, did not improve the process and basically similar maximum concentrations of hexanoic acid, of about 18 g/L, were obtained at each initial ethanol concentration; simply a larger unused amount of electron donor remained in the medium at the end of the process when its concentration was initially higher. Besides, near neutral pH values were optimal compared to slightly acidic or basic conditions. Slightly acidic conditions (*e.g.*, pH = 6.4) had a clear negative effect on bacterial growth and chain elongation with *C. kluyveri*. Instead, regulating the pH with an inorganic carbon source such as $NaHCO_3$, rather than simply using HCl/NaOH, had a somewhat positive effect on that chain elongation process. On the other hand, poorer culture media, *e.g.* without yeast extract, led to lower concentrations of end product, compared to reacher media.

A second set of, still on-going, experiments was setup in order to evaluate the bioconversion of syngas fermented media containing different ratios of acids and alcohols, at different pH values, showing the feasibility of such approach and reaching different efficiencies, depending on the characteristics of each fermented medium. Either mixed cultures[3] or pure cultures[109] can be used for chain elongation. A pure culture of *C. kluyveri* was used in the present study. Since C_1 gas fermenting acetogens are better producers of acids than alcohols, it appeared that the addition of exogenous ethanol may, occasionally, be useful or even necessary in order to ensure chain elongation at suitable alcohol/acid ratios. Besides, maintaining a near neutral, constant, pH value of the syngas fermented broth around 6.8 is useful for an optimal bioconversion process.

CONCLUSIONS: Chain elongation in *C. kluyveri* is most efficient with mixtures of both acetic and butyric acids as electron acceptors, with optimal conditions at near neutral pH and in a rich medium. Syngas fermented broth, containing any or both of those acids can then efficiently be used for chain elongation. A limited number of cultures will also generate ethanol from syngas fermentation, although its concentration may need to be adjusted, *i.e.* increased, in order to reach the required alcohol/acid ratios.

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Poster presentation: Medium Chain Fatty Acids Production Integrated with Continuous Biohydrogen Process: A Closed Loop Approach

Authors

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Abstract

Highlights

- MCFA production integrated with Continuous biohydrogen process
- Closed-loop biorefinery process to address the efficient carbon turnover
- Open culture bioaugmentation strategy for high rate MCFA production

Background

Rapid industrialization and urbanization is leading to massive solid waste generation and proper waste management practices are essential to avoid further environmental problems[110]. Currently, open dumping is the major practice of solid disposal followed by biomethanizaation and incineration. The biomethanization process produces CH_4 gas and it act as greenhouse gas. Amendment of biomethanization process produces the carbon-free hydrogen fuel along with volatile fatty acids (VFA: C_2-C_5) as a co-product. Separation of VFA from fermentation broth is challenging due to its high miscibility with waste, hence utilization of VFA as substrate in integrated process facilitates the economic viability and also addresses the disposal issues. Utilization of biohydrogen as reducing agent and volatile fatty acids (VFA: C_2-C_5) as carbon source for medium chain fatty acids (MCFA: C_6-C_{10}) production renders the sustainability and elevate the biomanufacturing sector as well.

Results and Discussion

The present study evaluated for the continuous biohydrogen production using food waste as feedstock and utilization of VFA as a substrate for medium chain fatty acids (MCFA: C_6-C_{10}) production in the secondary fermentation process. The

bioprocess performance was evaluated using enriched mixed microbial culture against the mixed culture augmented with *Clostridium kluyveri*. Wherein, augmentation with *C.kluyveri* induced the bioprocess performance and resulted the high MCFA yields than the corresponding mixed culture.

Conclusion

The study concludes the open culture bioaugmentation with *C.kluyveri* induces the process efficiency and results in high yields. The strategy could be used to elevate the process performance with minimal modifications. Also, the results enumerate the advantages of integrated multistage process for the production of Bio-H_2 and MCFA simultaneously addressing the food waste management issues in the biorefinery framework.

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