

RESEARCH

Open Access



Medium-chain carboxylates production from plant waste: kinetic study and effect of an enriched microbiome

Jerome Undiandeye^{1,2*}, Daniela Gallegos¹, Maria L. Bonatelli³, Sabine Kleinsteuber³,
Mohammad Sufian Bin-Hudari⁴, Nafi'u Abdulkadir^{5,6}, Walter Stinner¹ and Heike Sträuber³

Abstract

Background The need for addition of external electron donors such as ethanol or lactate impairs the economic viability of chain elongation (CE) processes for the production of medium-chain carboxylates (MCC). However, using feedstocks with inherent electron donors such as silages of waste biomass can improve the economics. Moreover, the use of an appropriate inoculum is critical to the overall efficiency of the CE process, as the production of a desired MCC can significantly be influenced by the presence or absence of specific microorganisms and their metabolic interactions. Beyond, it is necessary to generate data that can be used for reactor design, simulation and optimization of a given CE process. Such data can be obtained using appropriate mathematical models to predict the dynamics of the CE process.

Results In batch experiments using silages of sugar beet leaves, cassava leaves, and *Elodea*/wheat straw as substrates, caproate was the only MCC produced with maximum yields of 1.97, 3.48, and 0.88 g/kgVS, respectively. The MCC concentrations were accurately predicted with the modified Gompertz model. In a semi-continuous fermentation with ensiled sugar beet leaves as substrate and digestate from a biogas reactor as the sole inoculum, a prolonged lag phase of 7 days was observed for the production of MCC (C6–C8). The lag phase was significantly shortened by at least 4 days when an enriched inoculum was added to the system. With the enriched inoculum, an MCC yield of 93.67 g/kgVS and a productivity of 2.05 gMCC/L/d were achieved. Without the enriched inoculum, MCC yield and productivity were 43.30 g/kgVS and 0.95 gMCC/L/d, respectively. The higher MCC production was accompanied by higher relative abundances of Lachnospiraceae and Eubacteriaceae.

Conclusions Ensiled waste biomass is a suitable substrate for MCC production using CE. For an enhanced production of MCC from ensiled sugar beet leaves, the use of an enriched inoculum is recommended for a fast process start and high production performance.

Keywords Anaerobic fermentation, Caproate, Electron donors, Lactate, Microbial chain elongation, Waste biomass

*Correspondence:
Jerome Undiandeye
jerome.undiandeye@uniport.edu.ng
Full list of author information is available at the end of the article



Background

Even-numbered medium-chain carboxylates (MCCs) containing 6–12 carbon atoms are mainly produced as by-products of palm oil refining [1]. The demand for MCCs has been steadily increasing, and the global market size for these products is forecasted to reach USD 2.7 billion by 2027 [2]. MCCs have been reported to have a higher economic value than biogas, bioethanol, or biodiesel [3]. They are used in industries as feed additives [4], inhibitors of microbial corrosion [5] or for the production of lubricants [6]. A process commonly referred to as microbial chain elongation (CE), which is part of anaerobic fermentation, uses electron donors, usually ethanol or lactate, and electron acceptors such as acetate [7] for the production of MCCs, such as caproate (C6) and caprylate (C8). CE processes are catalyzed by anaerobic bacterial consortia and are basically redox reactions that follow the reverse β -oxidation pathway [8].

For a sustainable production of MCC, it is necessary to use substrates whose generation does not compete with food production. Such substrates include agricultural residues, such as sugar beet leaves or cassava leaves, as well as aquatic plants, such as *Elodea*. Sugar beet leaves are a by-product of sugar beet harvesting and account for 25–30% of the fresh sugar beet crop. At least 120 million tons of sugar beet leaves are produced annually in Europe [9], of which an estimated 18 million tons are produced in Germany. On the other hand, Nigeria produced 59.19 million tons of cassava in 2019 [10], accounting for 19.47% of the global cassava production in that year. One of the wastes usually generated during the harvesting of cassava root is cassava leaves, which can contain cyanide in high concentration [11], making them unsuitable for use as animal feed. *Elodea*, an invasive water plant, has been described as a global ecological problem that can often only be addressed by removal from water bodies [12]. The availability of these three kinds of plant waste makes them potential substrates for the production of MCCs by anaerobic fermentation, thereby reducing their potential environmental impact. Although these biomasses are easily degradable, they can be preserved by ensiling [13]. During ensiling, lactate and ethanol are formed that could be used as electron donors in subsequent MCC production [8], thus avoiding the need to add them to the process. Moreover, ensiling ensures year-round substrate availability and consistent quality, and serves as a pre-treatment technique [14].

For the start of a new MCC production process, the use of an inoculum can be helpful. An inoculum from a biogas reactor may be adequate for starting CE processes, and working under non-sterile conditions could reduce overall costs. However, the use of such an inoculum could be associated with a long lag-phase of CE and

limit the formation of MCCs [15] if the abundance of key community members, such as *Caproiciproducens*, which have been reported to correlate positively with caproate formation [16], is limited. One way to improve the CE efficiency could be the addition of broth from a previous or an already running CE process as co-inoculum or for bioaugmentation.

The yield and production rates of even- and odd-numbered carboxylates in an anaerobic fermentation process depend on the composition and dynamics of the microbial community, which in turn is influenced by the concentration and nature of the substrate, as well as process parameters, such as temperature and pH [7, 8]. Insight into the metabolic functions of community members with their various metabolic pathways enables the identification of process parameters that favor the production of specific MCCs. Using mathematical models, the understanding of the anaerobic fermentation process can be expanded. The models can provide kinetic parameters required for the design, operation, and optimization of processes in bioreactors to enhance their efficiency [17]. In addition, they can deliver information on the rate of a microbial process as a function of the operating parameters, enabling the determination of optimal conditions for the production of a specific MCC. While kinetic models for CE have been developed for pure cultures and model substrates, such as glucose [18], only a few studies on kinetic modeling of anaerobic fermentation of complex substrates by mixed microbial cultures have been reported.

The objectives of this study were to (i) investigate the suitability of selected silages for MCC production and determine kinetic parameters, (ii) study the effect of a mixture of an enriched microbiome and inoculum from a biogas reactor on the lag-phase of MCC production and the MCC yield, and (iii) identify the microbial community involved in the production of MCCs from ensiled sugar beet leaves using different inocula.

Materials and methods

Substrates and inocula

Four silages from different plant biomasses and one chemical standard were used as substrates. Sugar beet leaves were obtained from a local farm in Saxony, Germany, and ensiled as previously described [19]. Cassava leaves obtained from Ogoja, Southern Nigeria, were washed, grinded using a kitchen blender, packed into airtight bags and vacuum sealed. Silage consisting exclusively of *Elodea* biomass could not be prepared due to its high water content. Instead, a mixture of *Elodea* and wheat straw, ensiled as described by Gallegos et al. [20], was obtained from Deutsches Biomasseforschungszentrum (DBFZ). Maize silage was also obtained from the

DBFZ. Xylan (X) and lactate (L), which were used as chemical standard substrates, were obtained from Sigma Aldrich. The physico-chemical properties of all substrates, except xylan and lactate, are listed in Table 1.

Three different inocula were used for anaerobic fermentation. Inoculum 1 and 2 were obtained from two different anaerobic digesters at the DBFZ. Inoculum 1 (total solids, TS, 6.87%; volatile solids, VS, 72.18%TS; pH 7.57) was used for batch fermentation and originated from an anaerobic continuous stirred tank reactor (organic loading rate, OLR, 0.5 gVS/L/d; hydraulic retention time, HRT, 100 d; 38 °C) that was fed with a substrate mixture consisting of maize silage, cattle manure, and sun flower oil. Inoculum 2 (TS, 3.44%; VS, 70.23%TS; pH 7.0) was used for the semi-continuous fermentation of sugar beet leaf silage and was obtained from a full-scale biogas plant operated with a mixture of maize silage and cattle manure (HRT, 37 d; OLR, 4 gVS/L/d; 38 °C). Before being used, Inoculum 2 was sieved to remove large substrate particles using a mesh (2 mm). An enriched microbiome from the anaerobic fermentation of maize silage described in a previous study [21] was used as source for Inoculum 3. It was first centrifuged at 10,000×g for 10 min. The supernatant was then removed and the obtained pellet was washed with deionized water. The pellet obtained from 3 L of the enriched microbiome was resuspended in 1.2 L deionized water and used as Inoculum 3. All inocula were pre-treated for 1 h at 90 °C with the aim to inactivate methanogens.

Batch fermentation in serum bottles

Batch fermentations were set up in duplicates with silages of sugar beet leaves, cassava leaves, or *Elodea*/wheat straw mixture in identical 200 mL serum bottles with working volumes of 125 mL. The empty bottles were transferred into an anaerobic chamber, where

the substrates and Inoculum 1 were added. Each of the bottles contained 2.72 gVS of the respective substrate, 100 mL (4.91 gVS) of inoculum and, if necessary, anoxic deionized water to achieve the stated working volume. For comparison, bottles containing a standard plant substrate (maize silage) and bottles with a chemical standard substrate consisting of a mixture of xylan and lactate (xylan:lactate=3:1 w/w) were set up. Substrate-free control bottles containing only water and inoculum were also prepared. The pH in each bottle was adjusted to 7.57 ± 0.02 (the pH of the inoculum) using 10 M sodium hydroxide solution. The bottles were closed with butyl rubber stoppers and aluminium caps, removed from the anaerobic chamber, and incubated at 38 °C and 100 rpm for 28 days. To reduce the pressure in the bottles caused by gas production, each system was depressurized to $0.009 (\pm 0.002)$ bar(g) after each sampling.

Semi-continuous fermentation in reactors

Two 15-L stirred tank reactors (STR) named R7 and R8 (Bräutigam Kunststoffsysteme GmbH), each with a working volume of 12 L, equipped with an overhead stirrer (RZR2102, Heidolph Instruments) and operated in semi-continuous mode, were used for the anaerobic fermentation of sugar beet leaf silage in two trials. In the first experiment (E1), only Inoculum 2 was used, while a mixture of Inoculum 2 and Inoculum 3 was used in the second experiment (E2). Based on a previous study [22], the pH of the reactors was set at 5.50 ± 0.02 and then controlled automatically in both experiments using a feedback controller consisting of a pH sensor (pH 3110, WTW, Xylem Analytics Germany Sales GmbH & Co. KG), a control unit (Inpro 325X, Mettler Toledo) and a pump (Pumpdrive 5201, Heidolph Instruments) connected to each reactor and dispensing 10 M NaOH solution. The temperature was maintained at 38 °C (MA-4

Table 1 Mean values (\pm standard deviation) of the physico-chemical properties of substrates

Parameter	Unit	Substrates			
		MS	SBL	Cass	Elo
Total solids	%FM	38.90 \pm 3.68	16.14 \pm 0.16	24.29 \pm 0.07	24.17 \pm 0.10
Volatile solids	%TS	96.93 \pm 0.01	73.70 \pm 0.20	91.06 \pm 0.04	91.76 \pm 0.12
pH	–	3.86 \pm 0.01	4.71 \pm 0.22	4.76 \pm 0.04	3.94 \pm 0.01
Acetate	g/kgFM	77.56 \pm 0.00	42.93 \pm 0.01	26.73 \pm 0.00	56.14 \pm 0.00
Propionate	g/kgFM	8.73 \pm 0.00	0.22 \pm 0.00	ND	2.98 \pm 0.00
Butyrate	g/kgFM	5.64 \pm 0.00	0.09 \pm 0.00	0.12 \pm 0.00	1.45 \pm 0.00
Caproate	g/kgFM	0.16 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	ND
Lactate	g/kgFM	286.26 \pm 9.68	103.29 \pm 6.01	111.60 \pm 9.73	81.11 \pm 7.24
Ethanol	g/kgFM	10.07 \pm 0.00	15.32 \pm 0.04	23.83 \pm 0.01	0.65 \pm 0.00

MS, maize silage; SBL, sugar beet leaf silage; Cass, cassava leaf silage; Elo, *Elodea*/wheat straw silage; FM, fresh mass; TS, total solids; ND, not detected

Umwälzthermostat, Julabo). To compensate for pressure fluctuations during feeding, two 5-L gas bags were connected to the headspace of each reactor. The HRT was set to 4 days and the OLR was set to 21.9 gVS/L/d for both experiments.

For E1, the initial reactor content consisted of 6 L of Inoculum 2 and 3 L of substrate mixture. For E2, 3 L of each of Inoculum 2 and 3, and 3 L of substrate mixture were added to each reactor. For both E1 and E2, the headspaces of the reactors were flushed with N₂ to provide anaerobic conditions. Until the desired working volume of 12 L was reached, the reactors were operated in fed-batch mode without withdrawal of fermentation broth. Thereafter, 3 L of substrate mixture containing an appropriate amount of deionized water and substrate as well as 3 mL of trace element solution with a previously described composition [8] and 18 g of ammonium bicarbonate as additional nitrogen source (NH₄HCO₃; Carl Roth) were fed to each reactor daily. Before feeding, 3 L of fermentation broth was harvested from the reactors to keep the working volume constant.

Process analytics

TS and VS contents of substrates and fermentation broths were determined according to standard methods [23], and the values were corrected for the loss of organic acids and alcohols according to Weißbach and Strubelt [24] to avoid over-estimating the product yields and conversion degrees. The pH of samples was measured using a pH meter (model 3310; WTW, Xylem Analytics Germany Sales GmbH & Co. KG) equipped with a Sentix 41 pH electrode (WTW). Concentrations of carboxylates and ethanol were measured using a 7890A gas chromatograph with a flame ionization detector (FID; Agilent Technologies) as described by Apelt [25]. The head space of all reactors was sampled and the gas composition was measured with a gas chromatograph (Perkin Elmer) equipped with an auto-sampler following the method of Sträuber et al. [22].

To determine the gas production in the serum bottles, a digital manometer (LEO 5; Keller) connected to a filter (pore size 0.20 µm; Labsolute) was used to measure the relative pressure in the bottles. The amount of gas components produced in the bottles was calculated using the modified ideal gas equation shown in the following equation:

$$Gas_{(x_i)} (mmol) = \frac{P_{abs} \times \frac{Gas_{(x_i)}(\%)}{100} \times V_h}{R \times T} \times 1000 \quad (1)$$

where x_i is the gas component in the gas mixture, P_{abs} is the absolute pressure in the bottle (in mbar), V_h is the volume of the head space inside the bottle (75 mL), R is

the universal gas constant (83.140 mbar cm³/mol/K), and T is the gas temperature (K).

The gas production in the STRs was measured using Milligas counters MGC-IV3.1 (Ritter Apparatebau) connected to each reactor. The measured values were normalized to standard conditions as described previously [22]. Ammonia–nitrogen (NH₃–N) content in harvested fermentation broths from E1 and E2 was measured using ammonia test kits, Nessler reagent and a spectrophotometer (Hach DR 2000; HACH LANGE GmbH, Germany) according to the method described by Strach [26].

The yield of a fermentation product, P ($Y_{P/S}$; given in g_{Product} per kgVS_{Substrate}) in the batch systems and STRs was calculated using Eqs. 2 and 3, respectively. The selectivity of a carboxylate, P in both batch and STRs, was calculated using Eq. 4:

$$Y_{P/S} = \frac{\rho_P \times V}{m_S} \quad (2)$$

$$Y_{P/S} = \frac{\rho_P \times \vartheta}{m_s} \quad (3)$$

$$S_P = \left(\frac{\rho_P}{\rho_T} \right) \times 100\% \quad (4)$$

where ρ_P is the mass concentration of the product in the fermentation broth (g/L), V is the working volume in the bottles (=0.125 L), m_S is the mass of added substrate (kgVS), ϑ is the volume of fermentation broth daily withdrawn from the STRs (=3 L), S_P is the selectivity, and ρ_T is the total mass concentration of carboxylates in the fermentation broth (g/L).

Microbial community analysis

Cell pellets of the fermentation broths from E1 and E2 were collected at different timepoints and washed with 12 mM phosphate-buffered saline pH of 7.4 [27]. The cells were then stored at -20 °C until further processing. DNA was extracted from frozen pellets using the NucleoSpin Soil Kit (Macherey–Nagel). DNA quality was checked using a NanoDrop[®] ND-1000 UV–Vis spectral photometer (Fisher Scientific) and agarose gel electrophoresis; DNA concentration was determined with the Qubit dsDNA BR Assay-Kit (Invitrogen). Microbial community analysis was performed by amplicon sequencing of 16S rRNA genes. Library preparation and sequencing on the Illumina MiSeq platform were done as described by Logroño et al. [28].

Data were processed with DADA2 v.1.28.0 [29] to infer the amplicon sequence variants (ASVs). The R packages phyloseq v.1.44.0 and vegan v. 2.6.4 were used to perform the compositional and statistical analyses [30, 31]

on R software v.4.3.0 [32]. The SILVA database (version 138.1; [33]) was used for taxonomic assignment of ASVs. Raw sequence data were deposited at the NCBI database under the study accession PRJNA926909.

Modeling

Four kinetic models were used to describe the production of propionate, butyrate and caproate during the batch fermentation processes. These models were the first-order model given in Eq. 5, the modified Gompertz model given in Eq. 6, the Logistic model given in Eq. 7, and the Fitzhugh model given in Eq. 8, using the Solver add-in program in Microsoft Excel with sum of squared errors as an objective function:

$$\rho_t = \rho_{max}(1 - \exp(-kt)) \quad (5)$$

$$\rho_t = \rho_{max} \times \exp\left(-\exp\left(\frac{R_{max} \times e}{\rho_{max}}(\lambda - t) + 1\right)\right) \quad (6)$$

$$\rho_t = \frac{\rho_{max}}{1 + \exp\left[\frac{4 \times R_{max}(\lambda - t)}{\rho_{max}} + 2\right]} \quad (7)$$

$$\rho_t = \rho_{max} [1 - \exp(-kt)^n] \quad (8)$$

where ρ_t is the concentration of a given organic acid at timepoint t (g/L), ρ_{max} is the maximum possible concentration of a given organic acid (g/L), k is the first-order carboxylate production rate constant (/d), R_{max} is the maximum carboxylate production rate (g/L/d), λ is the process lag-phase (d), e is the Euler's constant, and n is the shape constant.

The model that fitted the experimental data best was identified using the coefficient of determination (R^2), the

Akaike Information Criterion (AIC) given in Eq. 9 and the root-mean-square-error given in Eq. 10:

$$AIC = N \times \ln\left(\frac{ss}{N}\right) + 2\nu \quad (9)$$

$$RMSE = \sqrt{\frac{ss}{N}} \quad (10)$$

where N is the number of experimental data; ss is the square sum of residuals; and ν is the number of parameters in the model.

All comparisons among substrates were performed using a one-way ANOVA followed by Tukey's post hoc test at 0.05 significance level using SAS v 10.0 software (SAS institute). Pearson correlation analysis was used to evaluate the interdependence of fermentation products using the same statistical software.

Results and discussion

Anaerobic fermentation in the batch systems

Batch fermentations for the production of short-chain carboxylates and MCC from silages of maize, sugar beet leaves, cassava leaves and *Elodea*/wheat straw, as well as from the model substrate xylan+lactate were carried out using Inoculum 1. The production yields and selectivities of acetate, propionate, butyrate and caproate were significantly different ($p < 0.05$) after 21 days (Table 2), probably due to the different physico-chemical properties of the individual substrates, in particular the contents of the electron donors lactate and ethanol (Table 1). Also, the proportion of lignocellulose and thus the accessibility and share of fermentable components such as hemicellulose varied between the different substrates (hemicellulose contents: 4.66%TS [34], 12.6%TS [35], 10.78%TS

Table 2 Maximum yields and selectivities (\pm standard deviation) of carboxylates after 21 days of batch fermentation

		Substrates				
		X+L	MS	SBL	Cass	Elo
Yield (g/kgVS)	C2	216.23 \pm 0.09	258.69 \pm 7.54	303.56 \pm 3.21	408.65 \pm 1.49	176.207 \pm 1.94
	C3	46.55 \pm 0.07	14.46 \pm 4.82	33.99 \pm 1.38	30.97 \pm 1.21	10.11 \pm 0.25
	C4	265.54 \pm 2.45	78.69 \pm 1.49	24.89 \pm 1.42	66.19 \pm 1.17	10.88 \pm 1.63
	C5	4.85 \pm 0.83	10.56 \pm 1.93	1.07 \pm 0.03	8.80 \pm 0.12	3.27 \pm 0.07
	C6	12.21 \pm 1.56	21.03 \pm 13.11	1.97 \pm 0.01	3.48 \pm 0.05	0.88 \pm 0.01
	Selectivity (%)	C2	51.69 \pm 1.77	66.09 \pm 7.74	85.90 \pm 0.79	81.73 \pm 3.69
C3		7.46 \pm 0.19	4.29 \pm 0.86	8.12 \pm 1.52	5.65 \pm 0.23	4.44 \pm 0.27
C4		34.47 \pm 0.94	13.48 \pm 0.41	5.28 \pm 0.36	10.65 \pm 0.18	3.89 \pm 0.03
C5		0.68 \pm 0.09	1.76 \pm 0.23	0.24 \pm 0.06	1.27 \pm 0.07	0.66 \pm 0.01
C6		1.60 \pm 0.14	3.39 \pm 1.96	0.42 \pm 0.01	0.68 \pm 0.02	0.18 \pm 0.01

C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate; X+L, xylate + lactate; MS, maize silage; SBL, sugar beet leaf silage; Cass, cassava leaf silage; Elo, *Elodea*/wheat straw silage

[13], and 28.3%TS [34] in *Elodea*, cassava leaves, sugar beet leaves, and wheat straw, respectively).

Acetate was the predominant fermentation product (83.00% from sugar beet leaves, 78.85% from cassava leaves, and 87.40% from *Elodea*/wheat straw), which is consistent with other studies [15, 36], where digestates from biogas reactors were also used as inoculum, probably due to the high abundance of acetate-forming bacteria in the inoculum.

MCCs such as caproate were not produced in any of the bottles until day 7 (Fig. 1). This was in accordance with Liu et al. [37], who reported that the time for caproate to be detected in batch fermentation of lactate can vary between 1 and 7 days, depending on the bacterial composition of the inoculum. The concentration of caproate increased gradually in all bottles, reaching a maximum of 0.64, 0.02, 0.05, 0.08, and 0.27 g/L in the systems with maize silage, *Elodea*/wheat straw silage, sugar beet leaf silage, cassava leaf silage and xylan + lactate, respectively, on day 21. Although enanthate (C7; 0.10 g/L) and caprylate (C8; 0.30 g/L) were produced in the bottles containing maize silage, the only MCC detected in all other bottles was caproate. Lactate and ethanol were completely consumed in all bottles, probably because they were used for the formation of carboxylates. According to Tang et al. [16], a high concentration of an electron donor like lactate can lead to increased butyrate and caproate production during anaerobic fermentation. Consequently, more caproate was produced from substrates with higher lactate concentration in the present study (Fig. 1). This emphasizes the need to ensure

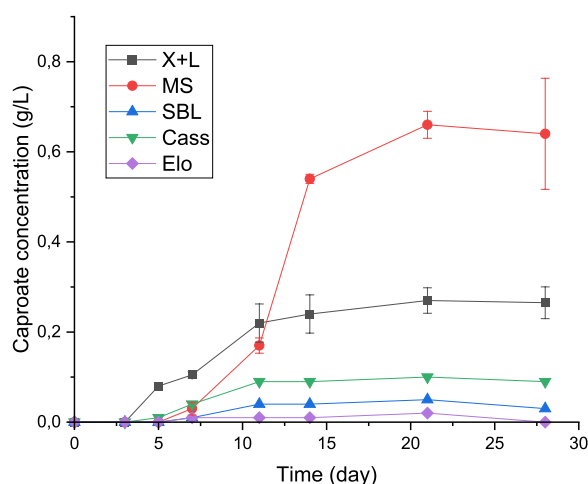


Fig. 1 Dynamics of caproate production from the batch fermentation of the substrates (X + L, xylate + lactate mixture; MS, maize silage; SBL, sugar beet leaf silage; Cass, cassava leaf silage; Elo, *Elodea*/wheat straw silage). Error bars show the standard deviation of biological replicates

proper ensiling of substrates that are to be used for MCC production. In the bottles with the chemical standard xylan + lactate, the caproate production remained low, despite high butyrate production (Table 2). It is likely that Inoculum 1 had low ability for microbial CE of butyrate or that the conditions were not suitable for this step.

A decrease in pH was observed in all bottles due to the formation of carboxylates (Figure S1). The pH drop was more pronounced during the first 3 days of fermentation, after which the pH remained relatively stable. This pattern was similarly observed by Fang et al. [38] during the anaerobic fermentation of mushroom residue. The pH decreased more in the order of systems containing silages of cassava leaves, sugar beet leaves and *Elodea*/wheat straw, which corresponded to the systems with higher carboxylate yields (Table 2), which was also observed by Eryildiz et al. [39].

Methane was not detected in any of the bottles in the first week, until traces of the gas were found in the bottles containing *Elodea*/wheat straw silage on day 7. On day 11, all other bottles except those with xylan + lactate contained methane. The gradual increase in methane production in the bottles indicates that the methanogens were only partially inactivated by the heat treatment of the inoculum or that methanogens were added with the non-sterile silages. The methane yields of the systems containing silages of cassava leaves, sugar beet leaves, *Elodea*/wheat straw and maize were 171, 196, 609 and 6 mL/gVS, respectively, on day 21. Of the systems with silages, those that produced more methane produced less caproate, indicating the competitive relationship between methanogenesis and CE.

In all bottles throughout the experimental period, the hydrogen partial pressure was below the value of 304 kPa required for a sustained caproate production [40], probably because the bottles were degassed after each analysis. The low hydrogen partial pressure may also have enhanced the excessive oxidation of ethanol to acetate [41].

Kinetics of carboxylate production in batch systems

Since the formation of propionate from lactate could be a competing pathway to the production of caproate by CE [39, 42], its kinetic parameters were determined in addition to those of butyrate and caproate production. The experimental values were fitted to four kinetic models, and various kinetic parameters were estimated using the Solver add-in program in Microsoft Excel. The estimated parameters and the fit of the measured data to the models are shown in Table 3 and Fig. 2, respectively. Most of the selected models adequately predicted propionate, butyrate and caproate production in all systems except in the bottles containing *Elodea*/wheat straw where only

Table 3 Kinetic and statistical parameters of carboxylate production from different silages determined by modeling

Model	Parameter	Cassava leaf silage			Sugar beet leaf silage		
		C3	C4	C6	C3	C4	C6
First order	k (/d)	0.115	0.129	–	0.123	0.119	–
	R ²	0.964	0.997	0.847	0.999	0.969	1.000
	AIC	– 46.529	– 50.542	– 54.341	– 57.995	– 36.484	– 123.724
	RMSE	0.048	0.038	0.030	0.024	0.090	0.000
	R _{max} (g/L/d)	0.016	0.021	0.005	0.017	0.011	0.006
Modified Gompertz	λ (d)	0.000	0.000	6.610	0.000	0.000	7.020
	R ²	0.945	0.996	0.993	0.989	0.9931	1.000
	AIC	– 41.318	– 53.028	– 86.243	– 52.771	– 48.147	– 152.317
	RMSE	0.059	0.028	0.004	0.029	0.038	0.000
Logistic	R _{max} (g/L/d)	0.023	0.037	0.008	0.027	0.018	0.013
	λ (d)	0.000	0.000	6.950	0.000	0.000	7.138
	R ²	1.000	1.000	1.000	1.000	1.000	1.000
	AIC	– 19.003	– 12.302	– 89.062	– 35.252	– 33.047	– 89.298
Fitzhugh	RMSE	0.238	0.361	0.003	0.086	0.099	0.003
	k (/d)	0.117	0.152	0.026	0.126	0.143	0.017
	n	0.321	0.387	2.471	0.428	0.473	2.519
	R ²	1.000	1.000	1.000	0.997	1.000	1.000
	AIC	– 22.384	– 26.659	– 68.128	– 53.608	– 31.549	– 92.298
RMSE	0.192	0.147	0.011	0.027	0.108	0.002	

C3, propionate; C4, butyrate; C6, caproate; k, first-order carboxylate production rate constant; R_{max}, maximum rate of carboxylate production; λ, lag phase; R², coefficient of determination; AIC, Akaike Information Criterion; RMSE, root-mean-square-error

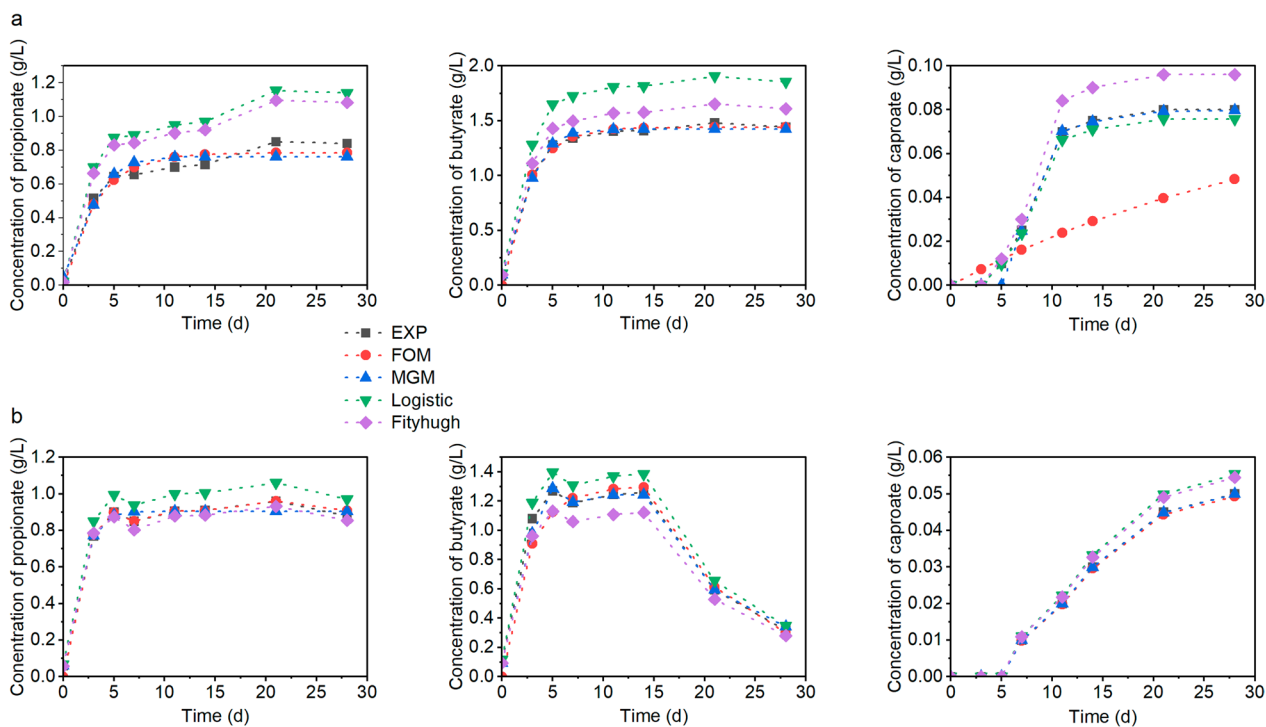


Fig. 2 Fitting curves from kinetic modeling of propionate, butyrate and caproate formation from the fermentation of silages of cassava leaves (a) and sugar beet leaves (b) using the first order model (FOM), the modified Gompertz model (MGM), the Logistic model and the Fitzhugh model

propionate production could be predicted by the models. The adequate prediction of the carboxylates production by the models is seen from the high values of R^2 , which were between 0.847 and 1.000. However, amongst the models used to describe the kinetics of propionate production, the first-order model gave the best prediction as indicated by the lowest values of AIC and RMSE. The model allowed for the estimation of a propionate production rate constant (k) of 0.115, 0.123 and 0.089/d in the system containing the silages of cassava leaves, sugar beet leaves and *Elodea*/wheat straw, respectively. These values were lower but not significantly different ($p > 0.05$) from 0.117, 0.126 and 0.103/d obtained from the Fitzhugh model for the silages of cassava leaves, sugar beet leaves and *Elodea*/wheat straw, respectively. Such discrepancy in kinetic parameters from different models could be attributed to the assumptions made in the derivation of the respective models. Morais et al. [43] and Coelho et al. [44] have also reported such differences in their studies. With lower AIC and RMSE values than the other models, the modified Gompertz model predicted butyrate production from the silages of cassava leaves and sugar beet leaves better than the other models. However, butyrate production from the system containing *Elodea*/wheat straw was too low to be predicted by any of the models. A lag phase of 0 days was predicted by both the modified Gompertz and the Logistic model for the production of propionate and butyrate from all substrates, with the shape factor of the Fitzhugh model being less than 1.0 for propionate and butyrate formation. A shape factor of less than 1 is an indication of the absence of a lag phase.

The formation of caproate from all other silages, except *Elodea*/wheat straw, was better described by the modified Gompertz model than by the other models. In fact, the carboxylate production could not be predicted by the first-order model. Lag phases of 7.02 and 6.61 days were estimated for caproate formation from sugar beet and cassava leaf silage, respectively. These estimates were consistent with the experimental data, where caproate was only detected from day 7, indicating that the modified Gompertz model adequately predicted the lag phases of these processes. The shape factor from the Fitzhugh model for caproate production from both cassava leaves silage and sugar beet leaves silage was greater than 1.0, which is an indication of the presence of a lag phase. The lag phases of caproate formation obtained in the present study are consistent with a lag phase of 6.905 days reported for caproate formation from swine wastewater using sludge from an anaerobic wastewater treatment plant as inoculum [43]. However, a lag phase of zero was reported for caproate formation from dairy wastewater using brewery wastewater as inoculum [44], indicating that the type of substrate and inoculum used

for fermentation influences the lag phase of carboxylate production. The lag phases for propionate and butyrate formation from all silages were zero, as confirmed by the experimental data, which was also observed by Morais et al. [43] and Coelho et al. [44]. In biotechnology processes, a short lag phase is often desired, since it is an indication of a fast start-up of fermentation processes.

The first-order production rate constant (k) is a measure of how fast a given compound is produced (or consumed) during a reaction [17]. For the bottles containing cassava leaf silage, k was higher for butyrate than for propionate, whereas the reverse was the case for sugar beet leaf silage. If the inoculum was adapted for the formation of MCC beyond caproate, odd-numbered MCC would have dominated the systems containing sugar beet leaf silage, while the systems containing cassava leaf silage would have been dominated by even-numbered MCC. Based on the k obtained, the formation of a desired MCC (odd- or even-numbered) can be quantified and maximized by altering certain conditions like reactant concentration, temperature and reactor type. Fogler [17] has reported that the selectivity equation (Eq. 11) can be used to maximize the formation of a desired product:

$$S_{D/U} = \frac{k_D}{k_U} c^{\alpha_D - \alpha_U} \quad (11)$$

where S is the instantaneous selectivity, k is the first-order production rate constant (/d), α is the positive reaction order, and c is the substrate concentration (g/L), D is the desired product, U is the undesired product.

Since the production of both propionate and butyrate was first-order, the selectivity reduces to the ratio of the first-order kinetic constants, indicating that either the temperature, pH, the type of substrates or the reactor type rather than the concentration of fermentation products (lactate and acetate) in the silages probably influenced the selectivity in the present study. Potential approaches to increase the selectivity of even-numbered MCC (as desired product) from the fermentation of sugar beet leaf silage could be operating at a lower pH or using a different type of reactor [17]. Similarly, the maximum carboxylate productivity R_{\max} was highest for butyrate in the fermentation of cassava leaf silage, whereas it was highest for propionate in the fermentation of sugar beet leaf silage, which is consistent with the measured concentrations of these two carboxylates. If operating parameters such as inoculum to substrate ratio and pH were optimized, higher values of R_{\max} and ρ_{\max} for MCCs could be achieved in batch fermentations of the substrates [8, 45]. As also noticed in Fig. 2, there was a sharp drop in butyrate concentration after 21 days during the fermentation of sugar beet leaf silage. Usually, butyrate concentration decreases, because the carboxylate is

elongated to caproate. However, in the present study, a corresponding increase in caproate concentration was not observed. It is therefore possible that the sharp drop in butyrate concentration in the system with sugar beet leaf silage was due to the conversion of the carboxylate to products other than caproate.

Carboxylate formation from sugar beet leaf silage in the semi-continuous systems

Although a higher yield of caproate was obtained from the fermentation of cassava leaves compared to sugar beet leaf silage, the latter was used as substrate for semi-continuous fermentation due to its availability. Two different experiments, E1 and E2, were conducted to determine the effect of the type of inoculum on MCC production. In the first trial, E1, where only Inoculum 2 was used, very low caproate production of up to 0.15 g/L was observed at the beginning until day 10 (Fig. 3A). The low MCC production in this period resulted from the type of inoculum rather than the low availability of electron donors for CE, as lactate and ethanol were always present in the harvested broth. Limited metabolic capabilities of the inoculum to completely utilize lactate and ethanol were similarly observed in the batch systems. Obviously, Inoculum 1 and Inoculum 2, both being digestates from biogas processes, were not well suited for a fast MCC production start. At several timepoints, the ethanol concentration was above the concentration in the input stream, indicating that this alcohol was produced in the system, which was similarly observed by Chwialkowska et al. [46] in the fermentation of acid whey. The high ethanol concentration may have inhibited caproate producing bacteria [47], resulting in the low initial caproate production.

Despite a substantial butyrate concentration of up to 4.99 g/L (see time-resolved butyrate curves in Figure S2), the caproate concentration remained below 1.0 g/L before day 50 (Fig. 3A). Thereafter, there was a sharp increase up to a maximum concentration of 2.41 g/L on day 74. The increased caproate concentration from day 50 was accompanied by a rapid drop in ethanol concentration, suggesting that ethanol presumably served as electron donor for the CE of butyrate and that microorganisms capable of this CE pathway had grown up.

The concentrations of enanthate and caprylate did not exceed 0.04 g/L throughout the fermentation period, which was similarly observed by Baleeiro et al. [48] in the fermentation of a mixture of lactate and acetate using an inoculum from the same biogas reactor as the one in the present study.

The volume of gas produced in the reactors fluctuated over time, with CO₂ being the major component, which is consistent with the study of Lambrecht et al. [21] on the fermentation of maize silage. CO₂ fluctuated between 66.82% and 90.78%, and H₂ between 6.85% and 28.55% throughout the experiment. Remarkably, only traces of methane (<1%) were detected in the gas phase of the reactors. The low methane concentration indicates that the methanogens were effectively inactivated by the heat treatment of the inoculum, unlike the batch processes where the methanogens were only partially inactivated or the inactivation was not permanent. The semi-continuous system with its shorter retention time facilitated slow-growing microorganisms to be washed out, whereas in the batch system the longer incubation time made it easier for methanogens to resettle. Traces of oxygen (up to 1.70%) were detected in the reactor

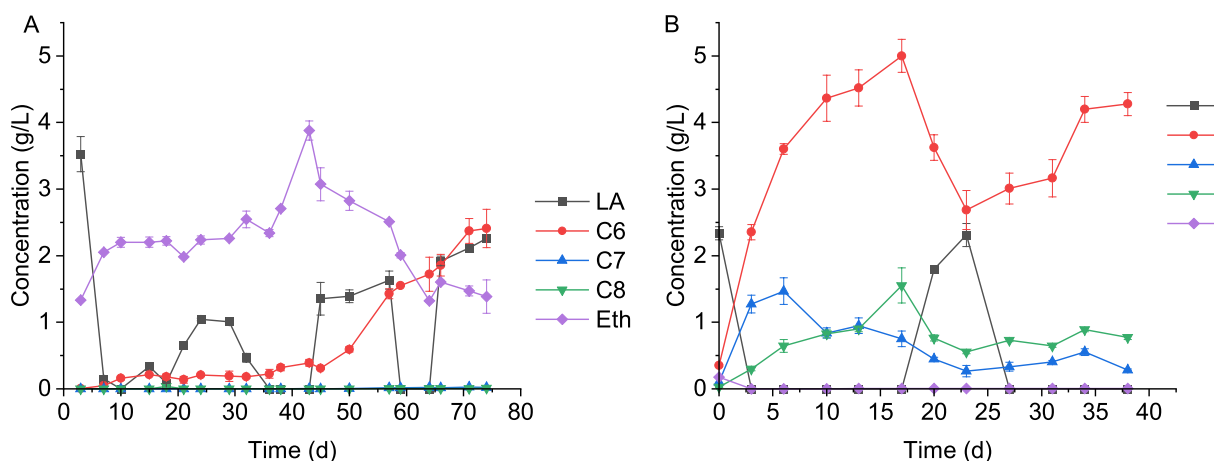


Fig. 3 Concentrations of MCCs, lactate and ethanol during the semi-continuous fermentation of sugar beet leaf silage in the experiments **A** E1 and **B** E2. Error bars represent standard deviations. LA, lactate; Eth, ethanol; C6, caproate; C7, enanthatate; C8, caprylate

headspace, which was probably caused by the daily feeding of the reactors with oxic substrate.

In the second experiment, E2, a mixture of Inoculum 2 and Inoculum 3 was used, the latter being an acclimatized enrichment culture capable of CE. The caproate concentration increased to 2.27 g/L after 3 days and continued to increase steadily over time until it reached a maximum of 5.0 g/L on day 17 (Fig. 3B). A lag phase, as observed in the batch systems and E1, was not present, probably because the microbial community (especially from Inoculum 3) quickly adapted to the conditions. Furthermore, the addition of the adapted Inoculum 3 resulted in ethanol and lactate being completely consumed in the start phase, unlike in E1. Between day 17 and day 31, there was a period of instability characterized by a drop in caproate concentration to 2.68 g/L and an increase in lactate concentration, probably due to a decrease in nitrogen availability resulting from the lack of addition of NH_4HCO_3 to the feed. Unlike in E1, enanthate and caprylate were produced in considerable quantities reaching a maximum of 0.75 and 1.55 g/L, respectively, on day 17.

The volume of gas produced also fluctuated over time, with a considerable proportion of methane of up to $30.04\% \pm 1.78\%$ formed during E2, probably because Inoculum 3 was more resistant to the thermal pretreatment. Hydrogen was mostly not detected, except on day 23 when it rose to 32.71%, at the same time the methane content was about 4% and the caproate concentration dropped. This inverse relationship of hydrogen content and caproate production was also reported by Sträuber et al. [49] for the anaerobic fermentation of maize silage and by Fang et al. [38] for the co-fermentation of mushroom residues and sewage sludge.

Yield and productivity

The maximum yields of fermentation products and productivities from the semi-continuous processes were calculated and summarized in Table 4. Clearly, the mixture of microbial communities from Inoculum 2 and Inoculum 3 enhanced the yield and productivity of MCCs from ensiled sugar beet leaves. Table 4 shows that the yields and productivities of MCC in E1 and E2 were significantly different ($p < 0.05$), indicating that CE was more efficient in E2 than in E1. The yield and productivity for MCCs (C6–C8) during E1 were 43.30 g/kgVS and 0.95 g/L/d, respectively, compared to 93.67 g/kgVS and 2.05 g/L/d, respectively, obtained during E2. The selectivity of caproate, enanthate and caprylate during E1 was 12.92, 0.11 and 0.04%, respectively, which was higher than in the batch fermentation, probably due to the adaptation of the microbial community enabled by the extended fermentation time and the semi-continuous

Table 4 Maximum product yields and productivities for carboxylates in the semi-continuous fermentation of sugar beet leaf silage in experiments E1 and E2

	Yield (g/kgVS)		Productivity (g/L/d)	
	E1	E2	E1	E2
Acetate	104.31	89.57	2.28	1.96
Propionate	11.87	5.72	0.26	0.15
Butyrate	38.62	37.82	0.85	0.83
Valerate	6.27	8.17	0.14	0.18
Caproate	42.74	60.92	0.94	1.33
Enanthate	0.40	10.06	0.01	0.22
Caprylate	0.17	22.69	Negligible	0.50

Table 5 Overview of maximum caproate concentrations from anaerobic fermentations of substrates with inherent electron donors for microbial CE

Substrate	Caproate concentration (g/L)	Reactor type	pH	References
Maize silage	3.10	STR	~ 5.0	[49]
Maize silage	6.12	STR	5.5	[21]
Grass silage	4.09	STR	5.5–6.2	[50]
Maize silage	1.40	Batch	~ 4.0	[8]
Sugar beet leaf silage	5.00	STR	5.5	This study
Sugar beet leaf silage	2.41	STR	5.5	This study
Food waste	10.00	Batch	NS	[51]
Acid whey	3.39	UAF	5.0	[52]

STR, stirred tank reactor; UAF, upflow anaerobic filter; NS, not stated

operation. During E2, the selectivity of caproate, enanthate and caprylate was 26.86%, 4.03% and 8.33%, respectively, further indicating the positive effect of the enriched microbiome, i.e. Inoculum 3, on the fermentation process. Unlike in the batch systems, the continuous systems produced higher yields of butyrate than propionate, which translated into higher yields of even-numbered MCC than odd-numbered MCC. This can be explained by differences in reactor type and operation as deduced from the selectivity equation (Eq. 8).

The substrates used in the present study contained electron donors for microbial CE, so that the addition of these components was not necessary. A comparison of the production of caproate from other substrates without the addition of external electron donors is shown in Table 5. The caproate concentration in the present study was in the same range as for the other substrates, indicating that sugar beet leaf silage is a potentially suitable

substrate for MCC production if an adapted inoculum is available.

Microbial community composition and dynamics during semi-continuous fermentation

The bacterial communities of the semi-continuous systems in experiments E1 and E2 were significantly different as revealed by 16S rRNA gene amplicon sequencing. Communities in E2 presented a higher diversity ($p < 0.05$) compared with E1, considering all timepoints and parallel reactors (Fig. 4A). Beta-diversity was visualized by NMDS (Fig. 4B), showing a clear separation between E1 and E2 samples as confirmed by PERMANOVA ($p < 0.001$). This result indicates that the use of Inoculum 3 in E2 significantly restructured the reactor microbiome. The parallel community dynamics in duplicate reactors R7 and R8 is also visible in the NMDS plot.

Inoculum 3 originated from the anaerobic fermentation of maize silage, in which the reactor microbiome was demonstrated to produce high titers of MCCs [21]. In contrast, Inoculum 2 was digestate from an anaerobic digester producing biogas. Therefore, the different community composition in E1 and E2 was expected. Since E2 was performed with a merged inoculum of Inoculum 2 and 3, it is clear that Inoculum 3 played a key role in structuring the community. Such bioaugmentation is a strategy used in anaerobic systems to obtain specific functions [53, 54]. Here, the success of bioaugmentation was reflected both in the caproate production and the microbial community composition.

Microbiomes in both experiments harbored mainly bacteria from the phylum Firmicutes, but the taxonomic composition was different on the genus level. In E1,

the predominance of *Caproiciproducens* was observed throughout the process (Fig. 5, Figure S3). In the beginning of the E1 process, also lactic acid bacteria of the genera *Lentilactobacillus* and *Lactobacillus* as well as ASVs assigned to *Clostridium* sensu stricto 12 were present in high relative abundance. Interestingly, between days 21 and 38, there was a shift in the community—the relative abundances of *Lentilactobacillus* and *Lactobacillus* first increased (until day 31) and then decreased considerably (below 5% abundance, Fig. 5). On day 31, *Caproiciproducens* was below 5% abundance, but after that, it remained to be dominant.

Caproiciproducens is known for its ability to produce caproate [55]. However, in E1, production of caproate was observed only after day 50, and the drop of ethanol concentration around the same time suggests that this was the electron donor for the chain elongation process (Fig. 3). As mentioned before, high ethanol concentrations may inhibit bacteria that can produce caproate. In particular, at ethanol concentrations higher than 4% (v/v), caproate production by *Clostridium kluyveri* decreased [47]. In experiment E1, the disturbance between days 21 and 38 may also have played a role in the adaptation of the community to produce caproate.

In E2, the bacterial community was more diverse and mainly composed of the genera *Shuttleworthia*, *Fastidiosipila*, [*Eubacterium*] *nodatum* group, *Lachnospira*, *Olsenella* and *Pseudoramibacter*. They belong to the clostridial families Lachnospiraceae and Eubacteriaceae (Firmicutes), except *Olsenella*, which is a lactic acid bacterium of the class Coriobacteriia (phylum Actinobacteriota). Representatives of Lachnospiraceae and Eubacteriaceae were predicted to convert lactate

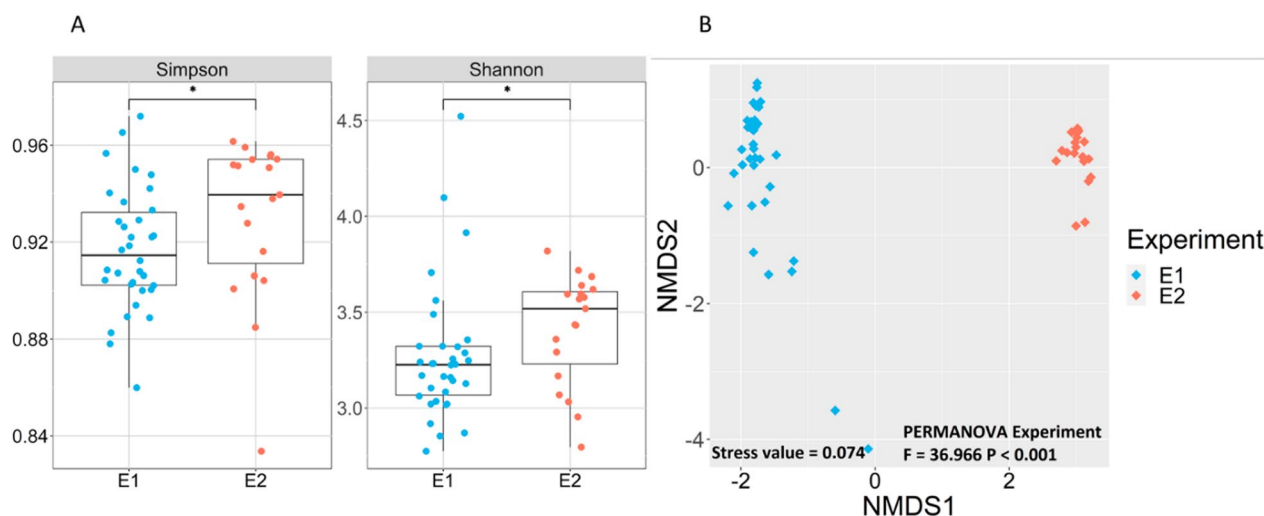


Fig. 4 Bacterial alpha- and beta-diversity in the semi-continuous systems E1 and E2. **A** Simpson and Shannon indices. Asterisks indicate significant difference ($p < 0.05$) based on Wilcoxon test. **B** Non-metric Multi-dimensional Scaling (NMDS) ordination based on Bray-Curtis distance

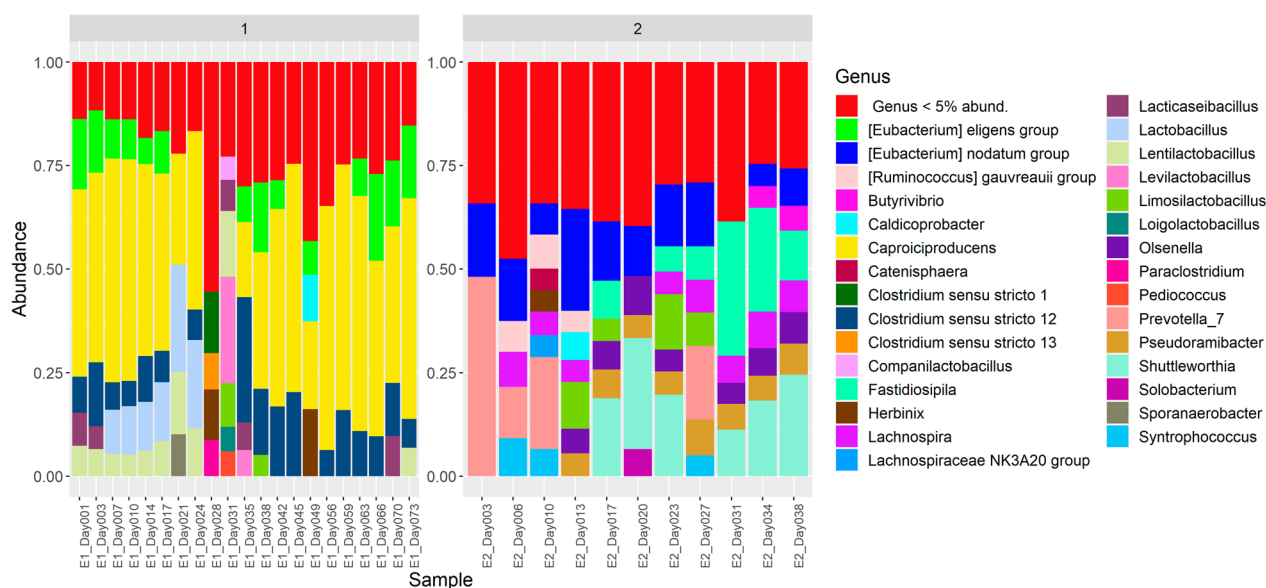


Fig. 5 Bacterial community composition of the semi-continuous systems E1 and E2. ASV data of the parallel reactors R7 and R8 were merged as not all sampling days were presented in the individual datasets (shown in Figure S3)

and acetate into MCC by integrative metagenomic, metatranscriptomic, and thermodynamic analyses of lignocellulosic ethanol fermentation conversion residue [56]. These authors specifically reported metagenome recovered genomes (MAGs) that clustered in a phylogenetic analysis with representatives of *Shuttleworthia* (Lachnospiraceae) and *Pseudoramibacter* (Eubacteriaceae). Also compared with Lambrecht et al. [21], the study that described the Inoculum 3 previously, many common genera were found.

Conclusions

Silages with a high content of electron donors such as lactate or ethanol for fueling the microbial CE are good substrates for feasible MCC production processes. In the present study, the modified Gompertz model predicted the formation of caproate from all silages better than the first-order, Logistic and Fitzhugh models. Besides shortening the start-up phase for the production of MCC, higher yields, selectivities and productivities can be achieved when using an enriched microbiome that has been tailored to microbial CE. When digestate from a biogas reactor was used as inoculum, the CE reactors were dominated by *Caproiciproducens*, while a more diverse community with Lachnospiraceae and Eubacteriaceae resulted when an enriched inoculum was used.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13068-024-02528-y>.

Additional file 1.

Acknowledgements

The authors thank the Petroleum Technology Development Fund (PTDF), Nigeria, and the German Academic Exchange Service (DAAD) for the PhD grant (57401043) offered to Jerome Undiandeye. Appreciation also goes to Ute Lohse of the UFZ and the laboratory staff of DBFZ for their technical assistance.

Author contributions

JU, WS, and HS conceptualized the study. JU and MH performed the experiments. JU, DG, MLB, MH, NA and HS analyzed the data. JU prepared the original draft. DG, MLB, SK, and HS revised the manuscript. All authors have read and approved the submitted manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL. Jerome Undiandeye was funded by Petroleum Technology Development Fund (PTDF), Nigeria, and the German Academic Exchange Service (DAAD) with a PhD grant (57401043).

Availability of data and materials

The raw sequence reads generated in the present study are deposited in the database of the National Centre for Biotechnology Information (NCBI) under the accession PRJNA926909.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Biochemical Conversion, DBFZ Deutsches Biomasseforschungszentrum Gemeinnützige GmbH, 04347 Leipzig, Germany. ²Department of Chemical Engineering, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria. ³Department of Microbial Biotechnology, Helmholtz Centre for Environmental Research – UFZ, 04318 Leipzig, Germany. ⁴Department of Isotope Biogeochemistry, Helmholtz Centre for Environmental Research – UFZ, 04318 Leipzig, Germany. ⁵Research Unit for Comparative Microbiome Analysis, Helmholtz Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany. ⁶Department of Microbiology, Sokoto State University, Sokoto 852101, Nigeria.

Received: 22 February 2024 Accepted: 4 June 2024

Published online: 12 June 2024

References

- Stamatopoulou P, Malkowski J, Conrado L, Brown K, Scarborough M. Fermentation of organic residues to beneficial chemicals: a review of medium-chain fatty acid production. *Processes*. 2020. <https://doi.org/10.3390/pr8121571>.
- Research and Markets. Global Medium Chain Triglycerides (MCT) Market Size, Share and Trends Analysis Report, 2020–2027. 2020. <http://www.researchandmarkets.com>. Accessed 28 Apr 2021.
- Chen W-S, Strik DPBTB, Buisman CJN, Kroeze C. Production of caproic acid from mixed organic waste: an environmental life cycle perspective. *Environ Sci Technol*. 2017;51:7159–68. <https://doi.org/10.1021/acs.est.6b06220>.
- Jackman JA, Boyd RD, Elrod CC. Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. *J Anim Sci Biotechnol*. 2020;11:44. <https://doi.org/10.1186/s40104-020-00446-1>.
- Churchward CP, Alany RG, Snyder LAS. Alternative antimicrobials: the properties of fatty acids and monoglycerides. *Crit Rev Microbiol*. 2018;44:561–70. <https://doi.org/10.1080/1040841X.2018.1467875>.
- Braune M, Yuan B, Sträuber H, McDowall SC, Nitzsche R, Gröngroft A. A downstream processing cascade for separation of caproic and caprylic acid from maize silage-based fermentation broth. *Front Bioeng Biotechnol*. 2021. <https://doi.org/10.3389/fbioe.2021.725578>.
- Wang H, Li X, Wang Y, Tao Y, Lu S, Zhu X, Li D. Improvement of n-caproic acid production with Ruminococcaceae bacterium CPB6: selection of electron acceptors and carbon sources and optimization of the culture medium. *Microb Cell Fact*. 2018;17:99. <https://doi.org/10.1186/s12934-018-0946-3>.
- Sträuber H, Bühligen F, Kleinsteuber S, Dittrich-Zechendorf M. Carboxylic acid production from ensiled crops in anaerobic solid-state fermentation—trace elements as pH controlling agents support microbial chain elongation with lactic acid. *Eng Life Sci*. 2018;18:447–58. <https://doi.org/10.1002/elsc.201700186>.
- Modelska M, Berłowska J, Kregiel D, Cieciora W, Antolak H, Tomaszewska J, et al. Concept for recycling waste biomass from the sugar industry for chemical and biotechnological purposes. *Molecules*. 2017. <https://doi.org/10.3390/molecules22091544>.
- FAO 2. Food and Agricultural Organization of the United Nations; Data of Crop Production. 2020.
- Umuhozariho MG, Shayo NB, Msuya JM, Sallah PYK. Cyanide and selected nutrients content of different preparations of leaves from three cassava species. *Afr J Food Sci*. 2014;8:122–9.
- Wang Y, Chen X, Liu J, Hong Y, He Q, Yu D, et al. Greater performance of exotic elodea nuttallii in response to water level may make it a better invader than exotic egeria densa during winter and spring. *Front Plant Sci*. 2019. <https://doi.org/10.3389/fpls.2019.00144>.
- Undiandeye J, Gallegos D, Lenz J, Nelles M, Stinner W. Effect of novel aspergillus and neurospora species-based additive on ensiling parameters and biomethane potential of sugar beet leaves. *Appl Sci*. 2022. <https://doi.org/10.3390/app12052684>.
- Undiandeye J, Kiman S, Abubakar AM, Dahunsi SO. Medium chain carboxylate production from cassava wastes pretreated by ensiling. *Biofuels Bioprod Biorefin*. 2023. <https://doi.org/10.1002/bbb.2485>.
- Bolaji IO, Dionisi D. Acidogenic fermentation of vegetable and salad waste for chemicals production: effect of pH buffer and retention time. *J Environ Chem Eng*. 2017;5:5933–43. <https://doi.org/10.1016/j.jece.2017.11.001>.
- Tang J, Dai K, Wang Q, Zheng S, Hong S, Zeng RJ, Zhang F. Caproate production from xylose via the fatty acid biosynthesis pathway by genus *Caproiciproducens* dominated mixed culture fermentation. *Biores Technol*. 2022. <https://doi.org/10.1016/j.biortech.2022.126978vol351>.
- Fogler HS. Elements of chemical reaction engineering. 5th ed. United States of America: Prentice Hall; 2016.
- Candry P, Van Daele T, Denis K, Amerlinck Y, Anderson SJ, Ganigue R, Arends JBA, Nopens I, Rabaey K. A novel high-throughput method for kinetic characterisation of anaerobic bioproduction strains, applied to *Clostridium kluyveri*. *Sci Rep*. 2018;8:9724. <https://doi.org/10.1038/s41598-018-27594-9>.
- Undiandeye J, Gallegos D, Sträuber H, Nelles M, Stinner W. Ensiling parameters in vertical columns and multiple kinetic models evaluation of biomethane potential of ensiled sugar beet leaves. *Biofuels*. 2022;13:995–1005. <https://doi.org/10.1080/17597269.2022.2059964>.
- Gallegos D, Wedwitschka H, Moeller L, Weirich S, Zehndorf A, Nelles M, Stinner W. Mixed silage of Elodea and wheat straw as a substrate for energy production in anaerobic digestion plants. *Energ Sustain Soc*. 2018;8:7. <https://doi.org/10.1186/s13705-018-0148-1>.
- Lambrecht J, Cichocki N, Schattenberg F, Kleinsteuber S, Harms H, Müller S, Sträuber H. Key sub-community dynamics of medium-chain carboxylate production. *Microb Cell Fact*. 2019;18:92. <https://doi.org/10.1186/s12934-019-1143-8>.
- Sträuber H, Bühligen F, Kleinsteuber S, Nikolausz M, Porsch K. Improved anaerobic fermentation of wheat straw by alkaline pre-treatment and addition of alkali-tolerant microorganisms. *Bioengineering*. 2015;2:66–93. <https://doi.org/10.3390/bioengineering2020066>.
- Strach K. Determination of total solids (dry matter) and volatile solids (organic dry matter). In: Liebetrau J, Pfeiffer D, editors. Collection of methods for biogas methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector. Leipzig, Germany: DBFZ; 2020. p. 50–6.
- Weissbach F, Strubelt C. Correcting the dry matter content of maize silages as a substrate for biogas production. *Landtechnik*. 2008;63:82–3.
- Apelt M. Determination of aliphatic, organic acids and benzaldehyde with headspace GC. In: Liebetrau J, Pfeiffer D, editors. Collection of methods for biogas methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector. Leipzig, Germany: DBFZ; 2020. p. 64–8.
- Strach K. Determination of the ammonia nitrogen content. In: Liebetrau J, Pfeiffer D, editors. Collection of methods for biogas methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector. Leipzig, Germany: DBFZ; 2020. p. 60.
- Baleiro FCF, Varchmin L, Kleinsteuber S, Sträuber H, Neumann A. Formate-induced CO tolerance and methanogenesis inhibition in fermentation of syngas and plant biomass for carboxylate production. *Biotechnol Biofuels Bioprod*. 2023;16:26. <https://doi.org/10.1186/s13068-023-02271-w>.
- Logroño W, Popp D, Kleinsteuber S, Sträuber H, Harms H, Nikolausz M. Microbial resource management for ex situ biomethanation of hydrogen at alkaline pH. *Microorganisms*. 2020. <https://doi.org/10.3390/microorganisms8040614>.
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581–3. <https://doi.org/10.1038/nmeth.3869>.
- McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*. 2013;8(4): e61217. <https://doi.org/10.1371/journal.pone.0061217>.
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solyoms P, Stevens M, Szöecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlenn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C,

- Weedon J. (2022) vegan: Community Ecology Package. R package version 2.6–4, <<https://CRAN.R-project.org/package=vegan>>.
32. R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.
 33. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Opens external link in new window. *Nucl Acids Res.* 41(1): 590–596.
 34. Gallegos-Ibanez D, Jurado-Molina J, Wedwitschka H, Delgado E, Nelles M, Stinner W. Ensiling of invasive *Elodea* sp., a novel aquatic biomass feedstock for the sustainable biogas production: effects of wheat straw and silage additives on silage quality and methane production. *ACS Agric Sci Technol.* 2023;3(6):477–86. <https://doi.org/10.1021/acsagscitech.3c00043>.
 35. Latif S, Romuli S, Barati Z, Müller J. CFD assisted investigation of mechanical juice extraction from cassava leaves and characterization of the products. *Food Sci Nutr.* 2020;8:3089–98. <https://doi.org/10.1002/fsn3.1517>.
 36. Sarkar O, Rova U, Christakopoulos P, Matsakas L. Influence of initial uncontrolled pH on acidogenic fermentation of brewery spent grains to biohydrogen and volatile fatty acids production: optimization and scale-up. *Biore Technol.* 2021;319: 124233. <https://doi.org/10.1016/j.biortech.2020.124233>.
 37. Liu B, Popp D, Müller N, Sträuber H, Harms H, Kleinstеuber S. Three novel clostridia isolates produce n-caproate and iso-butyrate from lactate: comparative genomics of chain-elongating bacteria. *Microorganisms.* 2020. <https://doi.org/10.3390/microorganisms8121970>.
 38. Fang W, Zhang P, Zhang T, Requeson DC, Poser M. Upgrading volatile fatty acids production through anaerobic co-fermentation of mushroom residue and sewage sludge: performance evaluation and kinetic analysis. *J Environ Manage.* 2019;241:612–8. <https://doi.org/10.1016/j.jenvman.2019.02.052>.
 39. Eryildiz B, Lukitawesa TMJ. Effect of pH, substrate loading, oxygen, and methanogens inhibitors on volatile fatty acid (VFA) production from citrus waste by anaerobic digestion. *Biore Technol.* 2020;302:122800. <https://doi.org/10.1016/j.biortech.2020.122800>.
 40. Angenent LT, Richter H, Buckel W, Spirito CM, Steinbusch KJJ, Plugge CM, et al. Chain elongation with reactor microbiomes: open-culture biotechnology to produce biochemicals. *Environ Sci Technol.* 2016;50:2796–810. <https://doi.org/10.1021/acs.est.5b04847>.
 41. Zagrodnik R, Duber A, Łężyk M, Oleskiewicz-Popiel P. Enrichment versus bioaugmentation—microbiological production of caproate from mixed carbon sources by mixed bacterial culture and *Clostridium kluyveri*. *Environ Sci Technol.* 2020;54:5864–73. <https://doi.org/10.1021/acs.est.9b07651>.
 42. Kucek LA, Nguyen M, Angenent LT. Conversion of l-lactate into n-caproate by a continuously fed reactor microbiome. *Water Res.* 2016;93:163–71. <https://doi.org/10.1016/j.watres.2016.02.018>.
 43. Morais NWS, Coelho MMH, Silva ADSE, Pereira EL, Leitão RC, Santos ABD. Kinetic modeling of anaerobic carboxylic acid production from swine wastewater. *Biore Technol.* 2020;297:122520. <https://doi.org/10.1016/j.biortech.2019.122520>.
 44. Coelho MMH, Morais NWS, Pereira EL, Leitão RC, Santos ABD. Potential assessment and kinetic modeling of carboxylic acids production using dairy wastewater as substrate. *Biochem Eng J.* 2020;156:107502. <https://doi.org/10.1016/j.bej.2020.107502>.
 45. Bevilacqua R, Regueira A, Mauricio-Iglesias M, Lema JM, Carballa M. Chain elongation may occur in protein mixed-culture fermentation without supplementing electron donor compounds. *J Environ Chem Eng.* 2022;10: 106943. <https://doi.org/10.1016/j.jece.2021.106943>.
 46. Chwiałkowska J, Duber A, Zagrodnik R, Walkiewicz F, Łężyk M, Oleskiewicz-Popiel P. Caproic acid production from acid whey via open culture fermentation—evaluation of the role of electron donors and downstream processing. *Biore Technol.* 2019;279:74–83. <https://doi.org/10.1016/j.biortech.2019.01.086>.
 47. Yan S, Dong D. Improvement of caproic acid production in a clostridium kluyveri H068 and Methanogen 166 co-culture fermentation system. *AMB Express.* 2018;8:175. <https://doi.org/10.1186/s13568-018-0705-1>.
 48. Baleeiro FCF, Kleinstеuber S, Sträuber H. Hydrogen as a Co-electron donor for chain elongation with complex communities. *Front Bioeng Biotechnol.* 2021;9:251. <https://doi.org/10.3389/fbioe.2021.650631>.
 49. Sträuber H, Lucas R, Kleinstеuber S. Metabolic and microbial community dynamics during the anaerobic digestion of maize silage in a two-phase process. *Appl Microbiol Biotechnol.* 2016;100:479–91. <https://doi.org/10.1007/s00253-015-6996-0>.
 50. Khor WC, Andersen S, Vervaeren H, Rabaey K. Electricity-assisted production of caproic acid from grass. *Biotechnol Biofuels.* 2017;10:180. <https://doi.org/10.1186/s13068-017-0863-4>.
 51. Nzeteu CO, Trego AC, Abram F, O’Flaherty V. Reproducible, high-yielding, biological caproate production from food waste using a single-phase anaerobic reactor system. *Biotechnol Biofuels.* 2018;11:108. <https://doi.org/10.1186/s13068-018-1101-4>.
 52. Xu J, Hao J, Guzman JLL, Spirito CM, Harroff LA, Angenent LT. Temperature-phased conversion of acid whey waste into medium-chain carboxylic acids via lactic acid: no external e-donor. *Joule.* 2018;2:280–95. <https://doi.org/10.1016/j.joule.2017.11.008>.
 53. Jiang J, Li L, Li Y, He Y, Wang C, Sun Y. Bioaugmentation to enhance anaerobic digestion of food waste: duration, frequency and economic analysis. *Biore Technol.* 2020;307: 123256. <https://doi.org/10.1016/j.biortech.2020.123256>.
 54. Li Y, Zhang Y, Sun Y, Wu S, Kong X, Yuan Z, Dong R. The performance efficiency of bioaugmentation to prevent anaerobic digestion failure from ammonia and propionate inhibition. *Biore Technol.* 2017;231:94–100. <https://doi.org/10.1016/j.biortech.2017.01.068>.
 55. Esquivel-Elizondo S, Bağcı C, Temovska M, Jeon BS, Bessarab I, Williams RBH, Huson DH, Angenent LT. 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. *Front Microbiol.* 2021. <https://doi.org/10.3389/fmicb.2020.594524>.
 56. Scarborough MJ, Lawson CE, Hamilton JJ, Donohue TJ, Noguera DR. Metatranscriptomic and thermodynamic insights into medium-chain fatty acid production using an anaerobic microbiome. *mSystems.* 2018. <https://doi.org/10.1128/mSystems.00221-18>.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.