



Selective bioelectrochemical purification of propionate from food waste hydrolysate

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ABSTRACT

Dark fermentation enables hydrogen production while generating propionate-rich streams from food waste. However, efficient separation of propionate from short-chain fatty acid (SCFA) mixtures remains challenging and costly in the downstream processes. Therefore, strategies enabling selective purification of propionate prior to separation from the fermentation broth are highly desirable. In this study, a bioelectrochemically assisted bench-scale process was developed as a proof-of-concept pretreatment to enhance selective purification of propionate prior to downstream processing. A single-chamber rotating disc bioelectrochemical reactor (RDBER) was used to selectively purify the hydrolysate via enhancing the consumption of butyrate by an electroactive biofilm of a recently published butyrate-oxidizing microbial consortium. In the RDBER, different hydraulic retention times (HRTs) without and with pH control were evaluated to investigate their effects on propionate fraction among SCFAs and process stability. The results show that with a pH of 7.0–7.3 and an HRT of 8 days, a propionate fraction of up to 95.5% can be achieved, with its concentration doubling compared to the feed. These results demonstrate the potential of this process as a pretreatment step prior to downstream separation, with the aim of reducing separation complexity and enhancing propionate recovery.

1. Introduction

According to United Nations Environment Programme [1], it is estimated that 1.05 billion tons of food were wasted worldwide in 2022, which is equivalent to 132 kg per capita per year. To manage this substantial organic waste, various technological approaches, such as dark fermentation (DF), have been explored and implemented to achieve environmentally friendly treatment while simultaneously enabling its valorization through the generation of hydrogen as an energy carrier and platform chemicals [2]. DF is carried out as partial anaerobic digestion without methanogenesis as the last step [3]. Consequently, H₂ is generated as the main product, while considerable amounts of short-chain fatty acids (SCFAs) remain in the hydrolysate [4]. To maximize energy and resource recovery from this process, additional technologies are often implemented following DF. For example, via a two-stage anaerobic digestion process (coupling DF and anaerobic digestion), CH₄ can be recovered from the remaining SCFAs [5]. Alternatively, the

SCFAs produced during DF, can be directly recovered for further applications or used as feedstock to produce other value-added and bio-based chemicals (e.g., plastics manufacturing and food additives) [5].

Propionic acid is a widely used SCFA, e.g. as an antimicrobial compound in food, anti-inflammatory agent, and herbicide [6]. In 2020, its production volume reached 470 kt, exhibiting an annual growth rate of 2.7%, and its market price ranged from 1250 to 1700 Euro per ton [7]. Beyond conventional petroleum-based production, intensive research efforts have increasingly focused on producing propionate from organic waste streams. For example, Yang et al. [8] reported that the proportion of propionic acid in the volatile fatty acids (VFAs, defined as SCFAs excluding lactic acid) could be increased to up to 70.74% by certain fermentation strategies. Similarly, Ali et al. [9] investigated SCFAs composition in the hydrolysate from a food waste surrogate and achieved a propionic acid fraction of approximately 55% of the total SCFAs. However, in both studies, substantial amounts of other SCFAs, particularly butyric acid and acetic acid, remained in the product stream. Such

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SCFA mixtures generally have a lower market value than individual acids and hence, efficient separation of single acids in their pure form is desirable [10]. Two key separation steps are usually involved: the recovery of SCFAs from the fermentation broth (separation from particles and from other dissolved organic carbon compounds) and the subsequent separation of individual acids from the mixture [11].

Currently, the recovery of SCFAs from fermentation broths largely depends on physicochemical separation technologies, such as membrane filtration, electrodialysis, adsorption, liquid-liquid-extraction and distillation [11,12]. These approaches have demonstrated high recovery efficiencies for total SCFAs and are effective in separating SCFAs from the fermentation broth. Nguyen et al. [13] have reported an average continuous SCFAs recovery of 73% from lignocellulosic biomass using an ultrafiltration-based system. Yousuf et al. [14] achieved a maximum removal efficiency of 74% for organic acids using a weak anion-exchange resin. However, the recovered SCFAs remain as a mixture, and the selective separation of individual SCFAs still is a major challenge due to similar structures and physicochemical properties. For example, adsorption-based processes often exhibit limited selectivity because acetic, propionic and butyric acids, all contain a carboxyl functional group [15,16]. Likewise, distillation is hindered by the relatively small differences in their boiling points (acetic acid: 118 °C; propionic acid: 141 °C; butyric acid: 162 °C) as well as the high energy demand associated with vaporizing substantial amounts of water present in the fermentation broth [17]. Therefore, it is of interest to explore strategies that selectively remove SCFAs other than propionate prior to conventional downstream separation processes, thereby enabling more efficient recovery.

Microbial electrolysis cells (MECs) are bioelectrochemical systems (BES) with the potential to sustainably produce hydrogen [18] (and/or methane) from various biomass waste streams (e.g. municipal wastewater) [19]. In a MEC, organic substrates like SCFAs are oxidized by an electroactive biofilm growing on the anode, e.g., in the case of acetic acid to produce CO₂, electrons and protons. The anodic substratum serves as the terminal electron acceptor. Via a power source, the electrons are further transferred to the cathode where they reduce the released protons to molecular hydrogen. By applying different biocatalysts (monoculture or mixed culture) and operating parameters, products like H₂ and after further archaeal conversion also CH₄ can be generated in the reaction chamber [20]. However, SCFAs are not equally favorable for the bacteria in the MECs, with acetate preferentially metabolized, followed by butyrate and finally propionate, which is often only poorly degraded [21]. This selective consumption makes propionate an ideal target product among the other SCFAs and enables its purification [22] while facilitating recovery of gaseous biofuels from the hydrolysate. Accordingly, efficient propionate purification depends on the removal of residual acetate and butyrate. *Geobacter sulfurreducens*, as the model electroactive bacterium, can effectively degrade acetate, whereas it barely uses butyrate. Recently, Elreedy et al. [23] enriched butyrate-oxidizing exoelectrogens from an anoxic sediment to increase the degradation efficiency in the treatment of butyrate-rich streams for hydrogen production. In the microbial consortium, the genus *Geobacter* (most likely *G. bemidjensis* and/or *G. bremensis* Dfr1), was presumably responsible for the syntrophic conversion of butyrate into acetate and H₂, while *Sporomusa* transformed H₂ and CO₂ into acetate. The addition of *G. sulfurreducens* to the consortium enhanced the conversion of acetate and H₂ [23]. Thus, this microbial consortium enables selective propionate purification from hydrolysate without requiring solvents or energy-intensive processes, while at the same time producing gaseous biofuels.

In this study, a two-stage bench-scale treatment and utilization of a food waste stream was implemented. A stirred-tank reactor was used to ferment food waste surrogate to produce a propionate-rich hydrolysate. A novel single chamber 10-L rotating disc bioelectrochemical reactor (RDBER) was then operated as a microbial electrolysis cell with the microbial consortium of Elreedy et al. [23] to selectively oxidize SCFAs

other than propionate. The aim of this study was to establish a proof of concept for a selective purification process that removes non-target SCFAs from the hydrolysate, resulting in a propionate-enriched stream with improved fraction - as a treatment step before downstream extractions of the SCFA from the fermentation broth. In the RDBER, different hydraulic retention times (HRTs) and pH conditions were applied to evaluate their impact on propionate fraction and yield.

2. Materials and methods

2.1. Hydrolysate characteristics

The hydrolysate was collected from a 15-L dark fermenter operated with vegan dog food as food waste surrogate (characteristics according to Ali et al. [24]) and pre-treated by means of sedimentation to separate large particles. Afterwards, only the supernatant was further filtered through filter paper 410 (VWR International, USA) with a particle retention of 2 µm. The hydrolysate was diluted tenfold due to high concentration of SCFAs and was autoclaved before being introduced to the RDBER.

2.2. Inocula and pre-culture conditions

The inoculum used in this study was a semi-synthetic consortium that comprised *G. sulfurreducens* PCA and a selectively enriched butyrate-oxidizing consortium from Elreedy et al. [23]. *G. sulfurreducens* was pre-cultivated anaerobically in BES-Medium (pH 7.4) with 20 mM sodium acetate and 40 mM fumarate as the electron donor and acceptor, respectively. Details regarding the minimal solution composition are available in an earlier publication [25] and supplementary material (SM). After cultivation for 3 to 7 days at 30 °C, the culture broth was centrifuged at 4600 rpm for 15 min and the cells were washed twice with salt solution (1×) under low-oxygen conditions. The 10-L RDBER was inoculated with *G. sulfurreducens* to reach an initial optical density at 600 nm (OD₆₀₀) of 0.1.

In order to cultivate and maintain the electroactive butyrate-oxidizing consortium for inoculation of the RDBER, an auxiliary 2-L single chamber bioelectrochemical system, as described earlier by Sturm-Richter et al. [26] with some modifications, was employed as a three-electrode setup with graphite-felt as the working electrode and platinum-mesh as the counter electrode. An Ag/AgCl reference electrode (Sensortechnik Meinsberg, Germany) was used, and the anodic potential was controlled at 0 mV against the standard hydrogen electrode (SHE) using a potentiostat (1010B, Gamry, USA). Before inoculation, small graphite pieces with an area of several cm² were attached to the anode via metal wires to be easily removed from the reactor after the cultivation. The reactor was filled with BES-medium containing 10 mM sodium butyrate as sole carbon source and electron donor instead of acetate and fumarate and omitting casamino acids or yeast extract. HEPES was replaced with 1.81 g L⁻¹ NaHCO₃ and 0.5 g L⁻¹ Na₂CO₃ as well. The medium was sparged with 20% CO₂ and 80% N₂ for at least 30 min. For inoculation, two graphite felts with active butyrate-oxidizing consortium obtained from the Gescher-lab (Hamburg University of Technology) were attached to the fresh anode in the 2-L single chamber reactor. *G. sulfurreducens* was added into this reactor as well to boost the degradation of acetate produced from butyrate degradation. The reactor was operated at 30 °C in a fed-batch mode, with an initial sodium butyrate addition of 10 mM. On day 12 (Run 1) the extra electrode felt pieces were detached from the anode and immersed into a sterile glass bottle with salt solution (1×). Biofilm was removed from the graphite felts via strong shaking and vortexing for 5 min and later, the liquid together with biofilm particles were inoculated into the 10-L RDBER. As mentioned above, the RDBER additionally was inoculated with *G. sulfurreducens*, leading to a final OD₆₀₀ of still around 0.1 of the two inoculation sources in sum. The 2 L reactor was still periodically fed with butyrate to keep the consortium viable. After 126 days, a new set of fresh

graphite felts were attached to the anode to produce the inoculum for Run 2, which was subsequently harvested 9 days later.

2.3. Microbial electrolysis cell (MEC) set-up and reactor operation

A single-chamber RDBER with 10-L construction volume was used in this study [25]. The RDBER is equipped with 14 graphite discs as working electrodes (anodes), reaching a total surface area of 10,000 cm² and, thus, a specific anode to reactor volume ratio of 100 m² m⁻³. The counter electrode consisted of a semi-cylindrical perforated stainless-steel plate that was located above the anode stack. The detailed configuration has been described in a previous publication [27].

The 10-L RDBER was operated as a MEC for two periods of 102 (Run 1) and 89 days (Run 2), respectively, both involving three phases: start-up batch phase, continuous operation phase, and an additional final batch phase. In both start-up batch phases, only butyrate was provided as organic substrate to facilitate the growth and development of the combined culture of *G. sulfurreducens* and the butyrate-oxidizing consortium.

This batch mode was then switched to a continuous mode feeding diluted hydrolysate at different HRTs. In Run 1, HRTs of 8 days, 4 days and 1 day were applied. Afterwards, in the final batch phase, the impact of different pH conditions (controlled at slightly acidic to neutral conditions (pH 6.0–6.6)) on propionate stability was investigated. The setups used in Run 1 and Run 2 are illustrated in SM. In Run 2, to isolate the influence of HRT, the pH value was controlled between 7.0 and 7.3. In the batch mode at the end of this cultivation, pH was increased to ~8 to 9. Details of operating parameters of both runs are given in [Table 1](#).

2.4. Process monitoring and analytical methods

The pH of the medium in the reactor was measured by a Ceralgel CPS71D pH sensor (Endress+Hauser, Germany) and data was initially recorded every five minutes with a Liquiline CM448 automatic acquisition system (Endress+Hauser, Germany). Along with the incorporation of the pH control, the pH was adjusted with 5 M NaOH and 10% HCl with the help of a Biostat® A (Sartorius, Germany). The curves of pH and current density development were smoothed via the Lowess method (span = 0.05) to remove noise caused by e.g. sampling or other short flow disruptions [28].

During operation, liquid samples were taken from the reactor three to four times a week and filtered through 0.45 μm polyethersulfone (PES) membrane filters. SCFAs, including acetic acid, lactic acid, propionic acid, butyric acid and valeric acid, were quantified via a Metrohm 881 Compact Pro Ion Exchange Chromatograph (Metrohm, Switzerland) equipped with a Metrosep Organic Acids 250/7.8 column. Every sample was measured in duplicate, and the average of the two measurements was used as the representative value of that sample. Duplicate measurements were performed to assess and minimize analytical variability associated with sample preparation and instrumental measurement.

These analytical replicates should not be considered independent reactor replicates. Dissolved organic carbon (DOC) was measured for selected samples using a total organic carbon (TOC) analyzer (TOC-LCPH/CPN, Shimadzu, Germany). The recirculation outlet and reactor outlet were both located on the rear flange of the reactor, while the sampling port was positioned within the recirculation loop. The reactor liquid was recirculated at a rate corresponding to approximately one cycle every 20 min. Therefore, samples collected from the recirculation loop were considered representative of the reactor effluent and used as the outflow concentrations in subsequent calculations.

Gases produced in the reactor chamber were emitted through the gas outlet, which was connected to a gas flow meter (MilliGascounter®, Ritter, Germany) and further collected in a gas sampling bag. The gas mixture was measured and analyzed in a 490 Micro GC (Agilent Technologies, Germany) with two different carrier gases (argon and helium) and two stationary phases (CP-Molsieve 5 Å Plot and J&W PoraPLOT U GC columns, Agilent Technologies, Germany). Measurements were repeated two to three times.

2.5. Microbial sequencing analysis

Anodic and cathodic biofilm samples were collected at the end of Run 2 by scratching off the biomass on the electrode surfaces. Planktonic cells were harvested from the reactor effluent by centrifugation at 4600 rpm for 15 min, washed with 1× salt solution and subsequently resuspended in the same salt solution. Genomic DNA was extracted from the samples using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Amplicon sequencing of the 16S rRNA gene was performed by IMA GmbH (Karlsruhe, Germany) on an Illumina MiSeq platform using paired-end sequencing (2 × 250 bp reads). The primer pairs Bakt_341F/Bakt_805R [29] and A519F/A906R [30,31] were used for the amplification of bacterial and archaeal 16S rRNA genes, respectively.

Sequence processing and taxonomic assignment were performed using the PipeCraft workflow [32], including quality filtering, chimera removal, clustering of operational taxonomic units (OTUs) at 97% sequence similarity, and taxonomic classification against the SILVA SSU Ref NR 99 database (release 138.2).

2.6. System performance characterization

Recorded current was normalized to the total anode surface area of the reactor and represented as current density ($\mu\text{A cm}^{-2}$). Coulombic efficiency (*CE*, given in %) is the ratio between the electrons observed in the current (n_{clt}) and the electrons released from consumed organic acids (n_{oa}), and was calculated as follows:

$$CE (\%) = \frac{n_{cu}}{n_{ca}} \times 100 = \frac{1000^* \int_0^t Idt}{F^* Z^* V^* \Delta c} \times 100 \quad (1)$$

where I (A) is the recorded current over time t (s), F (C mol^{-1}) is the Faraday constant with the value of 96,485, Z ($\text{mol e}^{-} \text{mol}^{-1}$ substrate)

Table 1
Operating parameters of the two runs in the RDBER.

Parameters		Run 1			Run 2	
Temperature		30 °C			30 °C	
Medium volume		8.4 L			9.4 L ^a	
Initial OD ₆₀₀		~ 0.1			~ 0.1	
Electrode potential		Day 0–7 300 mV vs. SHE ^b Day 7 until end 100 mV vs. SHE			100 mV vs. SHE	
Operating mode	Batch	Continuous	Batch	Batch	Continuous	Batch
Substrate	Butyrate	Hydrolysate	Hydrolysate	Butyrate	Hydrolysate	Hydrolysate
HRT	–	8 d; 4 d; 1 d	–	–	6 d; 4 d; 8 d	–
pH control	–	–	pH 6.0–6.6	–	pH 7.0–7.3	pH 8.0–9.0
Operating time	11 days	27 days	64 days	13 days	64 days	12 days

^a Increased working volume is attributed to addition of a mixing vessel in the reactor periphery for micro-aeration tests (see chapter 3.2.1).

^b 300 mV was applied during the first 7 days in an attempt to facilitate biofilm growth.

refers to the theoretical number of electrons released from the complete oxidation of one mole of organic acids (8 for acetate), V (L) denotes the reactor liquid volume, and Δc (mmol L⁻¹) is the change in organic acid concentration over time period t .

Net reaction rates (r , given in mmol L⁻¹ d⁻¹) including net production (or net gain) (+), and net loss (−) rates (both of which may reflect changes in hydrolysate SCFA concentrations), of the five SCFAs were calculated at each sampling time point according to the following equations for the batch (eq. 2) and continuous (eq. 3) operation modes, respectively:

$$r(\text{batch}) = \frac{c_{i,t_x} - c_{i,t_{x-1}}}{\Delta t} \quad (2)$$

where c_{i,t_x} (mmol L⁻¹) is the concentration of organic acids i in the reactor at sampling time point t_x , Δt (d) is the time interval between two consecutive sampling time points t_x and t_{x-1} .

$$r(\text{conti}) = \frac{c_{\text{out},i,t_x} - c_{\text{in},i,t_x}}{HRT_{t_x}} + \frac{c_{\text{out},i,t_x} - c_{\text{out},i,t_{x-1}}}{\Delta t} \quad (3)$$

where c_{out,i,t_x} and c_{in,i,t_x} represent the concentrations of organic acids i in the outflow and inflow of the reactor at sampling time point t_x , $c_{\text{out},i,t_{x-1}}$ denotes the concentration of organic acids i in the reactor outflow at the previous sampling time point t_{x-1} , HRT_{t_x} (given in d) represents the hydraulic retention time applied at sampling time point t_x .

The average net propionate production rate ($\overline{NPPR}$) for each HRT phase was calculated as the mean of the propionate reaction rates during that phase.

The total mass of organic acids (T_m , given in mmol) in the inflow and outflow, as well as the average propionate volumetric output rate ($\overline{PVOR}$) during the continuous mode were calculated according to the following equations:

$$T_m = Q^* \int_{t_1}^{t_2} c_{\text{in},i} dt, \text{ or } T_m = Q^* \int_{t_1}^{t_2} c_{\text{out},i} dt \quad (4)$$

$$\overline{PRR} = \frac{Q \times \int_{t_1}^{t_2} c_{\text{out},\text{prop}} dt}{V \times (t_2 - t_1)} \quad (5)$$

where Q is the volumetric flow rate (L d⁻¹), $c_{\text{out},i}$ and $c_{\text{in},i}$ are the concentrations of organic acids i in the outflow and inflow, respectively, and t_1 and t_2 denote start and end of the duration of a certain hydraulic retention time.

The propionate fraction among SCFAs (PF , given in %) represents the ratio between propionate concentration (c_{prop}) and the sum concentration of five SCFAs (c_{SCFAs}) (acetate, lactate, propionate, butyrate and valerate).

$$PF = \frac{c_{\text{prop}}}{c_{\text{SCFAs}}} \times 100 \quad (6)$$

The propionate enrichment factor (PEF) is defined as the ratio between the propionate concentrations in the inflow and outflow, respectively.

$$PEF = \frac{c_{\text{out},\text{prop},t_x}}{c_{\text{in},\text{prop},t_x}} \quad (7)$$

Gas production rate (GPR , given in L_{gas} L_{liquid}⁻¹ d⁻¹) was calculated from the cumulative gas volume recorded by the gas flow meter, normalized to the corresponding measurement period and liquid volume.

$$GPR = \frac{V_{\text{gas},t_2} - V_{\text{gas},t_1}}{V \times (t_2 - t_1)} \quad (8)$$

where V_{gas,t_1} and V_{gas,t_2} are the gas volumes recorded by the gas flow meter at the beginning and end, respectively, of the corresponding HRT period.

For the reactor samples, the phase-averaged value and variability (standard deviation) of each parameter were calculated as follows, based on the sampling points within each HRT phase.

$$\bar{X}_i = \frac{1}{n} \sum_{j=1}^n X_{ij} \quad (9)$$

$$SD_i = \sqrt{\frac{\sum_{j=1}^n (X_{ij} - \bar{X}_i)^2}{n-1}} \quad (10)$$

where X is the measured or calculated quantity (e.g., concentration, net reaction rate, current density, or gas fraction), i represents different SCFAs or gases (where applicable), j represents the sampling point and n is the number of sampling points within the HRT phase.

The error bars of the phase-averaged values represent temporal variability within a given HRT phase rather than experimental reproducibility. The measurement data of SCFA concentrations and DOC, together with the number of observations during continuous operation in both runs, are provided in the supplementary excel file.

For the hydrolysate fed, the average and the variability of the SCFA and DOC concentrations in each phase were calculated using time-weighted values to account for the different durations for which each feeding bottle was used during each HRT phase. The calculations for the feedings were as follows:

$$\bar{c}_{TW} = \sum_{j=1}^n c_j \frac{t_j}{T} \quad (11)$$

$$SD_{TW} = \sqrt{\sum_{j=1}^n (c_j - \bar{c}_{TW})^2 \frac{t_j}{T}} \quad (12)$$

where $\bar{c}_{TW}$ is the time-weighted mean concentration, c_j is the concentration in the bottle j , t_j is the duration for which bottle j was used, T is the total operation time of an HRT phase, n is the number of feeding bottles, SD_{TW} is the time-weighted standard deviation.

3. Results and discussion

3.1. Run 1: Identification of suitable operational parameters

In a previous study, Elreedy et al. [23] successfully enriched a butyrate-oxidizing consortium and demonstrated its capacity for butyrate and acetate removal from the hydrolysate. However, the study was conducted in a small-scale reactor (250 mL working volume) and was not specifically designed for propionate purification. Consequently, the effects of key operational parameters, such as HRT and pH, as well as the long-term stability and performance of the consortium, remain largely unexplored. Therefore, Run 1 was conducted as a long-term experiment in a 10-L reactor to evaluate the robustness of the enriched consortium under more practical conditions and to identify suitable operational parameters for propionate purification.

3.1.1. Effect of HRT and resulting pH conditions on propionate purification

Prior to continuous operation with slightly acidic hydrolysate, the reactor was initiated with a batch phase of 11 days, during which butyrate was supplied as the sole carbon source for acclimatization and establishment of the butyrate-oxidizing consortium. After 10 days, only approximately 1 mmol L⁻¹ butyrate (~9% of the initial concentration) was consumed (Fig. 1(a)). This can be attributed to the low initial inoculum, as the consortium had been harvested from several graphite felt pieces, each providing only a few cm² of surface area, resulting in a limited initial biomass concentration in the 10-L reactor.

After the start-up phase, the reactor was continuously fed with diluted SCFAs-rich hydrolysate obtained from DF of food waste

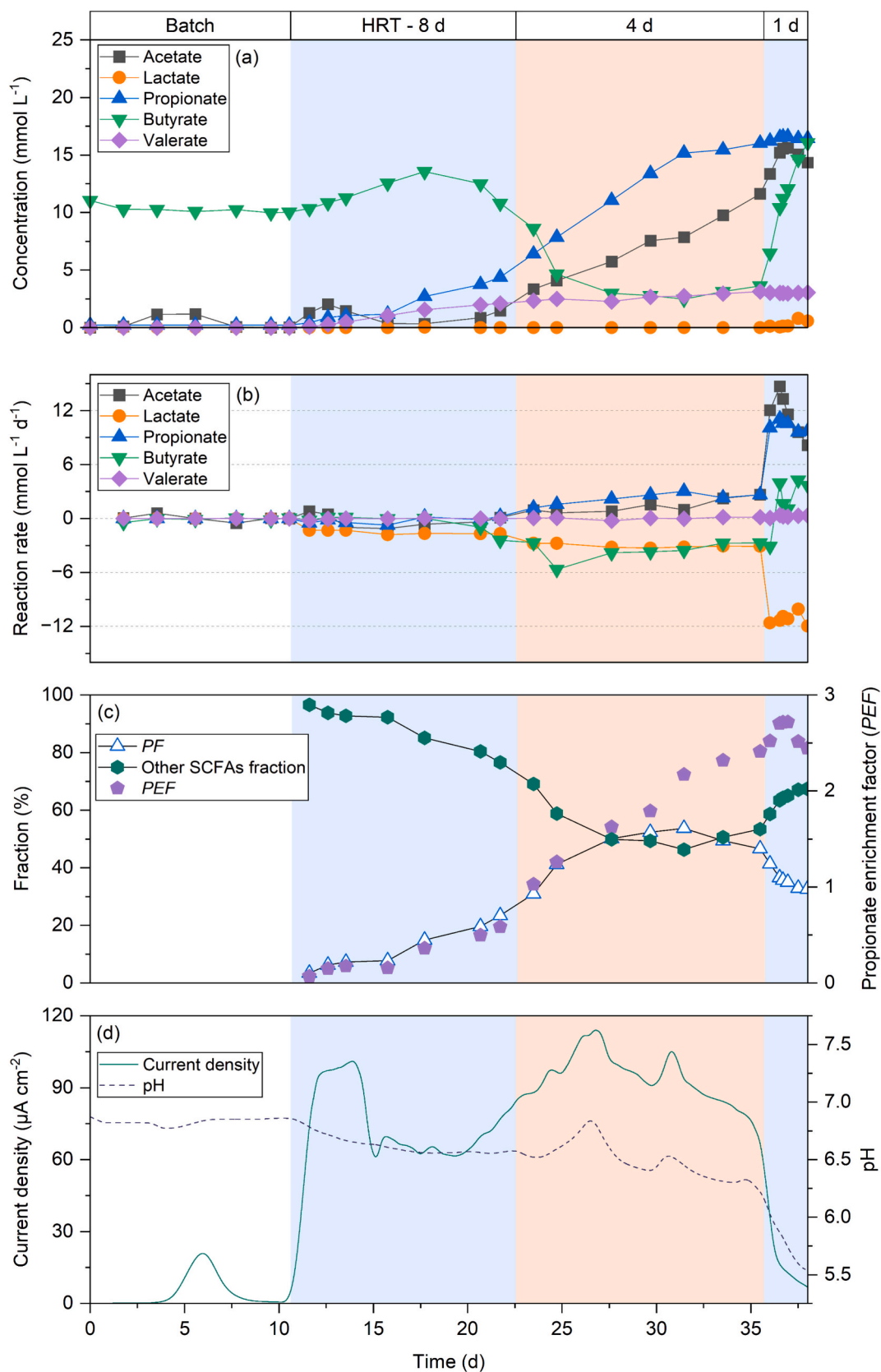
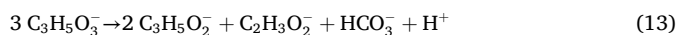


Fig. 1. (a) Development of SCFAs (acetate, lactate, propionate, butyrate, and valerate) during the first two operation phases of Run 1 (first 38 days), (b) corresponding SCFAs net reaction rates (r ; +: net production rate; -: net loss rate), (c) propionate fraction among SCFAs (PF), other SCFAs fraction and propionate enrichment factor (PEF), and (d) current generation and pH variation.

surrogate. During the three tested phases of different HRTs, the hydrolysate contained butyrate, lactate, propionate, acetate, and valerate, with total SCFAs concentrations of 42.7 ± 4.9 , 42.2 ± 2.7 and 40.0 ± 1.4 mmol L⁻¹, respectively. Among these acids, butyrate was the predominant component, indicating that the DF can be characterized as a butyrate-type fermentation [33].

At an HRT of 8 days, the total SCFA concentration decreased by 63% to an average concentration of 15.9 ± 2.7 mmol L⁻¹. Butyrate remained the dominant SCFA, whereas the propionate fraction (PF) increased to approximately 23% by the end of the 8-day HRT (Fig. 1(c)). Further shortening the HRT to 4 and 1 days resulted in average PF-values of $46.1 \pm 7.8\%$ and $35.7 \pm 3.2\%$, respectively. It is noteworthy that at an HRT of 4 days, the propionate enrichment factor (PEF) continued to increase, whereas the PF peaked at 53.8% and subsequently decreased. This behavior can be explained by the accumulation of propionate and acetate, which significantly lowered the pH and reduced the current density (as shown in Fig. 1(d)). In this study, *G. sulfurreducens* was co-inoculated into the system, with acetate serving as the main substrate for current generation, but it was shown that bacterial growth is significantly inhibited at a pH of 6.4 and completely suppressed at a pH of 5.5 [34]. Although reducing the HRT to 1 day increased the PEF to 2.6 ± 0.1 , the overall system performance was compromised. The enhanced accumulation of acids further decreased the pH to below 6 and down to 5.5, leading to complete inhibition of butyrate degradation (Fig. 1(b)). As mentioned in the introduction, *G. bemidjensis* and/or *G. brementis* Dfr1 are presumably the key species involved in the conversion of butyrate to acetate and hydrogen. Previous studies [35,36] have reported that *G. bemidjensis* exhibits optimal growth at a pH of 7.0, whereas the optimal growth pH range for *G. brementis* Dfr1 is between 5.5 and 6.7. Therefore, the decrease in pH to 5.5 may have adversely affected the growth and activity of these microorganisms, thereby limiting butyrate degradation and PF.

In contrast, propionate generation appeared to be less affected by pH than butyrate degradation, as the PEF continued to increase despite the substantial decrease in pH. Fermentatively, propionate can be generated via the succinate, acrylate and propanediol pathways, with lactate contributing directly through the acrylate pathway and indirectly via the CO₂-dependent succinate pathway after oxidation to pyruvate [37]. Lactate degradation can also yield 1,2-propanediol, which serves as a precursor for propionate formation [38]. Collectively, these mentioned pathways highlight lactate as a central substrate for propionate generation. Accordingly, the overall stoichiometric equation, where three lactate molecules are transformed into two propionate molecules and one molecule of acetate, can be written as follows:



On the basis of this reaction and the degradation of one butyrate molecule into two acetate molecules, the contribution of lactate to propionate formation was quantified via a mass balance analysis for the HRT of 1 day. At this HRT, the degradation of SCFAs was largely suppressed, resulting in a less complex system and enabling a more reliable evaluation of lactate conversion. As shown in Table 2, acetate production reached 93.5% of the theoretical maximum expected from lactate and butyrate conversion. However, the observed propionate production exceeded the theoretical maximum attributable to lactate conversion alone, indicating that additional non-SCFA compounds likely

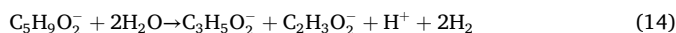
contributed to propionate formation. SCFAs in the hydrolysate fed during this phase accounted for $50.7 \pm 2.6\%$ of the DOC (as shown in SM), whereas the remaining fraction was likely composed of complex sugars, amino acids, and long-chain fatty acids. These compounds may serve as potential precursors for propionate production, as it was shown, for example, for amino acids [39].

In summary, increasing PF depends not only on the mineralization of other SCFAs but also on the concurrent generation of propionate. The contribution of the electrode to propionate enrichment is indirect. The anodic biofilm was established using a culture previously enriched for butyrate oxidation, and its activity in the present reactor is therefore expected to contribute to the preferential removal of butyrate through electrochemically associated microbial metabolism. In contrast, acetate does not necessarily rely on anodic oxidation for its removal and can be consumed through methanogenesis. The addition of *G. sulfurreducens* was intended to enhance the conversion of intermediate products (acetate and H₂) from butyrate oxidation. The propionate itself is generated fermentatively from lactate and non-SCFA precursors, as reflected in the mass balance in Table 2. The reactor provides a reducing redox environment in the bulk phase, a condition under which fermentative metabolism favors reduced products such as propionate [40]. Since lactate conversion to propionate is only slightly affected by acidic conditions, the limiting step for achieving high PF is the mineralization of non-propionate acids, mainly butyrate and acetate. However, these two processes prefer near-neutral pH conditions. Thus, Run 2 was operated under approximately neutral conditions via in-situ pH control, and the HRTs were set between 4 and 8 days as well.

3.1.2. Effect of acidic conditions on propionate stability

Following continuous operation, the reactor was run in batch mode to investigate the recovery of the biofilm after unfavorable exposure to an acidic shock at the HRT of 1 day and to evaluate the stability of propionate. As shown in Fig. 2(a), acetate consumption persisted even at pH values below 6.0. The consumption of acetate gradually increased the pH, allowing the medium to recover toward neutral conditions (Fig. 2(b)). During Phase I (day 38.0 to 43.5), the Coulombic efficiency (CE) calculated based on the net acetate consumption was approximately 46%, indicating the exoelectrogens remained active following the acidic shock. However, more than 50% of the electrons were not transferred to the electrode, suggesting alternative electron sinks, such as methanogenesis. This interpretation is supported by the methane and hydrogen fractions of 58–59% and 5%, respectively, detected on days 38.5 and 44.5. Notably, substantial butyrate degradation occurred only after the pH increased above 6.0, suggesting that maintaining the pH above 6.0 is essential for sustaining system performance.

The propionate concentration exhibited a two-step increase in the absence of lactate. During phase I (Fig. 2(a)), propionate increased by approximately 8%, providing further evidence that non-SCFA compounds contributed to propionate production. Subsequently, from day 55 to day 71, the increase in the propionate concentration closely corresponded to the decrease in valerate concentration, yielding a ratio of 0.85. As described in Eq. 14 from Wang et al. [41], this observation suggests that valerate may have contributed to propionate formation.



The stable concentration and high fraction (> 95%) of propionate

Table 2

Mass balance of SCFAs (excluding valerate) at the HRT of 1 day in Run 1. M and A denote the theoretical maximum and actual production, respectively.

Compound	Total inflow (mmol)	Total outflow (mmol)	Propionate production (mmol)	Acetate production (mmol)
Butyrate	287.55	218.63	–	137.84 (M)
Lactate	229.42	6.04	148.92 (M)	74.46 (M)
Acetate	86.15	284.71	–	198.56 (A)
Propionate	124.54	320.09	195.55 (A)	–
Conversion efficiency (%)			131.3%	93.5%

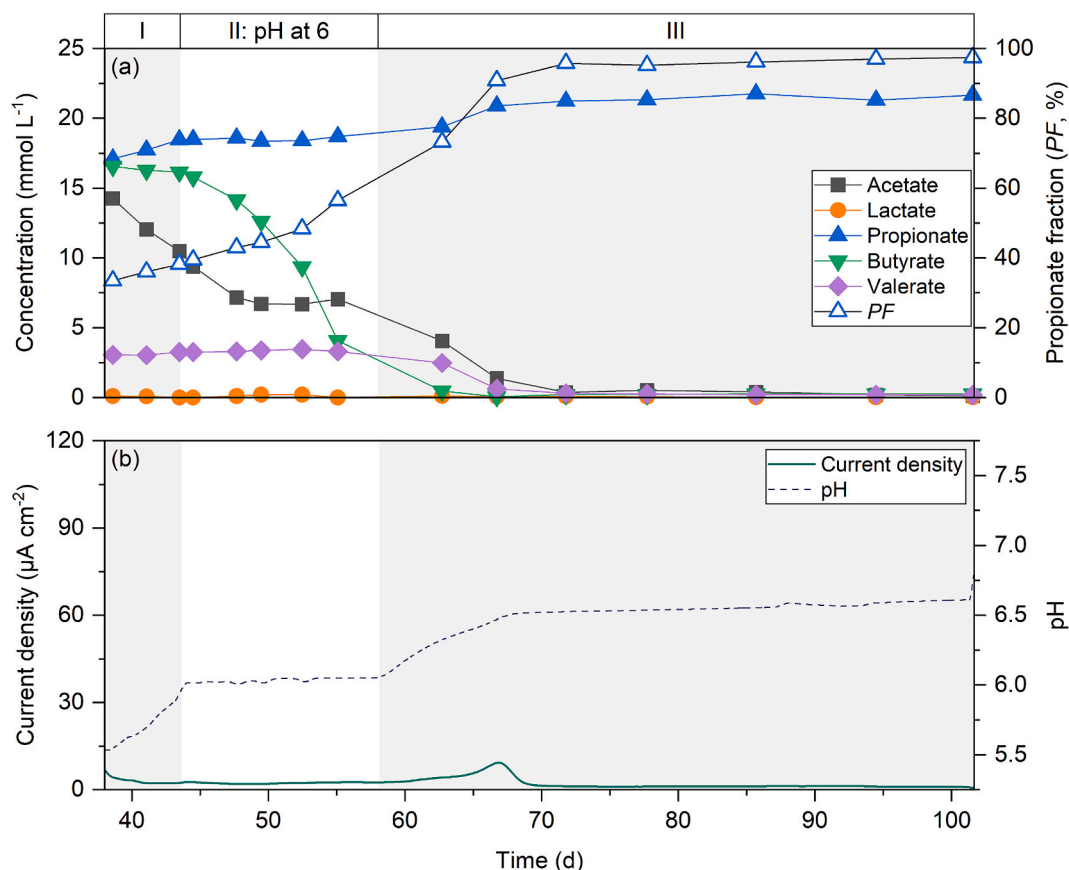


Fig. 2. (a) Development of SCFAs and propionate fraction among SCFAs (PF) in the final batch phase of Run 1 (day 38 to day 102), and (b) corresponding current generation and pH variation. The pH was maintained at 6 during phase II, whereas no pH regulation was applied during phases I and III.

suggest that there was no appreciable net loss of propionate at pH values between 6.0 and 6.6, despite the presence of methanogenic activity.

3.2. Run 2: Process optimization and enhanced purification performance

To ensure stable system performance while minimizing the addition of chemicals, the second long-term operation (Run 2) was optimized with pH regulation at 7.0 shortly after the onset of continuous operation. The pH control was activated only when the pH fell below 7.0.

3.2.1. Effect of HRT under pH control

In Run 2, the start-up phase lasted 13 days. During the first 9 days, only a 12% decrease in propionate was observed, as shown in Fig. 3(a), similar to the batch phase of Run 1. However, after only four more days, the butyrate oxidation finally reached 84% in Run 2. Two reasons might be responsible for this difference. First, it seems that the prolongation of the batch phase allowed sufficient growth of the butyrate-oxidizing consortium. In addition, although the inocula were obtained from the same 2-L reactor, the inoculum of Run 2 was harvested 135 days later. Thus, the ongoing cultivation of the consortium in the 2-L reactor with intermittent butyrate feeding potentially led to further enrichment of the butyrate-oxidizing consortium.

It has to be mentioned that in the subsequent continuous operation, at an HRT of 4 days, four short micro-aeration events of 30 min each were carried out within 9 days to evaluate the inhibiting effect of oxygen dosing on methanogenesis (Fig. 3(d)). As this is not the focus of the present study, the corresponding details are not discussed, and data collected from the first aeration event to 24 h after the final aeration event were excluded from the further evaluation of this HRT.

The hydrolysate supplied during the three HRT phases in Run 2 contained total SCFA concentrations of 36.3 ± 8.6 mmol L⁻¹, $50.0 \pm$

7.6 mmol L⁻¹ and 51.9 ± 3.8 mmol L⁻¹, respectively. Under these conditions, the PF increased to more than 40% at an HRT of 6 days, while it reached $61.8 \pm 10.1\%$ and $91.8 \pm 3.0\%$ during the subsequent two HRT phases (Fig. 3(c)).

However, since PF was still increasing at the end of the 6-day HRT and had not reached a steady state or peak, direct comparisons are not appropriate. Nevertheless, it is evident that increasing the HRT from 4 to 8 days enhanced acid degradation and further increased PF by 30%. In contrast, the PEF remained mostly around 2 throughout the continuous operation independent of HRTs. Occasional higher values were attributed to the delayed response of the reactor to changes in influent propionate and lactate concentrations resulting from continuous operation with long HRT, as shown in Fig. 3(c). Comparing PF and PEF of Run 2 with the ones achieved in Run 1, these results demonstrate improved system performance under neutral conditions. Notably, at the HRT of 8 days, a maximum propionate fraction of 95.5% was achieved, with an average exceeding 90%. During the later phase of 8-day HRT period, the propionate fraction decreased slightly to 87.5%, mainly attributed to the absence of lactate and the lower propionate concentration in the hydrolysate. Nevertheless, even when the butyrate concentration in the hydrolysate increased from approximately 16 mM to 33 mM, it was still completely degraded in the reactor, as shown in Fig. 3(a) and (b), allowing a relatively high propionate fraction to be maintained. Overall, during the 8-day HRT period, the DOC concentration in the hydrolysate decreased from 3903 ± 356 to 1365 ± 195 mg L⁻¹, corresponding to a 65.0% decrease, while the proportion of SCFAs in the DOC increased from $53.2 \pm 4.0\%$ to $74.5 \pm 8.1\%$ (see SM). These changes suggest that, in addition to SCFAs, non-SCFA organic compounds were also converted during the process.

Although propionate accumulation was desirable in this study, increasing propionate concentrations may potentially affect the

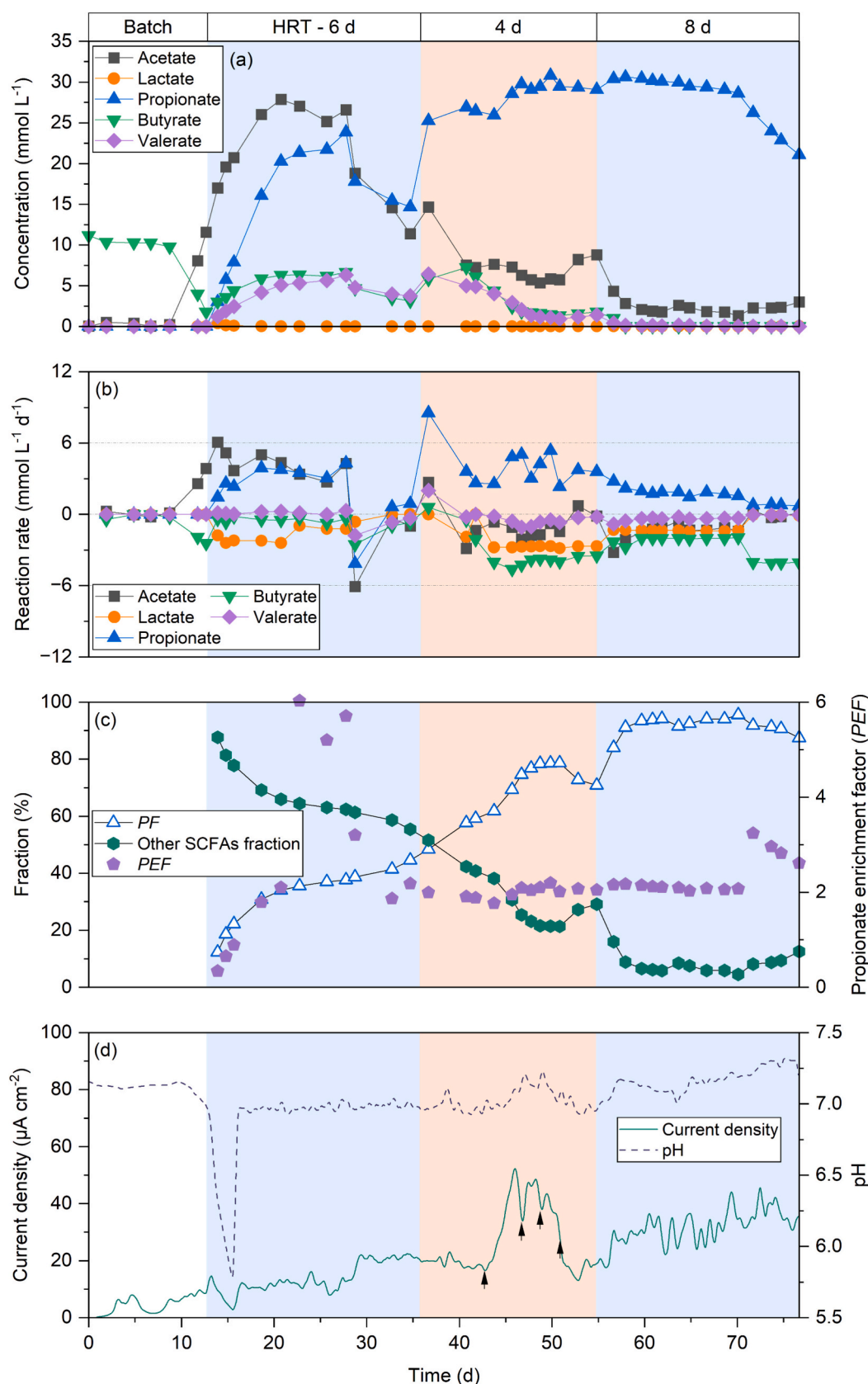


Fig. 3. (a) Development of SCFAs in the first two operating phases of Run 2 (first 77 days), (b) corresponding SCFAs net reaction rates (r), (c) propionate fraction among SCFAs (PF), other SCFAs fraction and propionate enrichment factor (PEF), and (d) current generation and pH variation. Arrows indicate the time points at which micro-aeration was applied for 30 min. For the evaluation of system performance at the HRT of 4 days, data collected from the first micro-aeration event until 24 h after the final micro-aeration event were excluded from the analysis.

conversion of propionate and non-targeted SCFAs via electroactive and methanogenic pathways. For example, Hari et al. [42] observed 20% lower propionate removal at 36 mM compared with 4.5 mM propionate in the propionate-fed MECs, indicating that increasing propionate concentrations may limit its conversion and thus favor its accumulation. In addition, Härrer et al. [43] reported a 27% decrease in the specific growth rate of planktonic *G. sulfurreducens* with addition of 25 mM propionate, suggesting that elevated propionate concentrations may potentially affect the electroactive microorganisms and, consequently the conversion of non-targeted SCFAs through the electroactive pathways. From the methanogenic perspective, the accumulation of propionate has been associated with lag phases in methane generation and further acidification with higher organic loading rates [44]. Therefore, the increasing propionate concentration in the present study may influence both electroactive and methanogenic conversion of non-targeted SCFAs and propionate itself. However, such effects may have been limited in the present study, as the reactor was operated under near-neutral pH conditions, and the maximum propionate concentration was approximately 30 mmol L⁻¹.

3.2.2. Effect of alkaline conditions on propionate stability

Before the start of batch mode at the end of Run 2, reactor medium was partially replaced with fresh hydrolysate (pH adjusted to above 8) as the reactor was formerly operated at an HRT of 8 days which resulted in low SCFAs concentrations and microbial activity may have been limited. Under alkaline conditions, propionate degradation was detected when pH exceeded 8.7 (Fig. 4(a)). Syntrophic propionate oxidation is widely regarded as a rate-limiting step in anaerobic digestion because of its unfavorable thermodynamics and its reliance on tightly coupled interactions between syntrophic propionate-oxidizing bacteria (SPOB)

and hydrogen- and/or formate- utilizing hydrogenotrophic methanogens [45,46] as well as acetoclastic methanogens [47]. Among these, the continuous scavenging of hydrogen and formate to maintain their concentrations at sufficiently low levels is particularly critical for overcoming the thermodynamic barrier to propionate oxidation [48]. In this study, propionate degradation is likely, at least partially, associated with the activity of electroactive microorganisms, as current production persists during the degradation process, albeit at a lower level, as shown in Fig. 4(b). Hari et al. [42] investigated electron flow during propionate oxidation in MECs and identified the simultaneous occurrence of multiple electron transfer pathways, including acetate/H₂ and acetate/formate-mediated routes, with current production serving as the main electron sink. Although it has been claimed that propionate could be directly oxidized to generate current, this process requires specific exoelectrogens such as *Geobacter subterraneus* [49], which were not detected in the present study. Further investigations are needed to identify the propionate oxidation pathways within this consortium. Although the mechanisms governing propionate oxidation remain unclear, the low degradation rate of 0.8 mmol L⁻¹ d⁻¹ observed under alkaline conditions, together with the absence of net propionate loss at mildly acidic pH, suggests that substantial net propionate degradation is unlikely under the usual operational conditions around neutral pH. Hence, in order to purify hydrolysate and maximize the recovery of propionate, the pH conditions should be controlled between 6 and 8.

Before terminating the final batch phase, butyrate, lactate and valerate were spiked into the reactor to verify that the microbial consortium remained metabolically active prior to the final sampling of biofilms and planktonic cells.

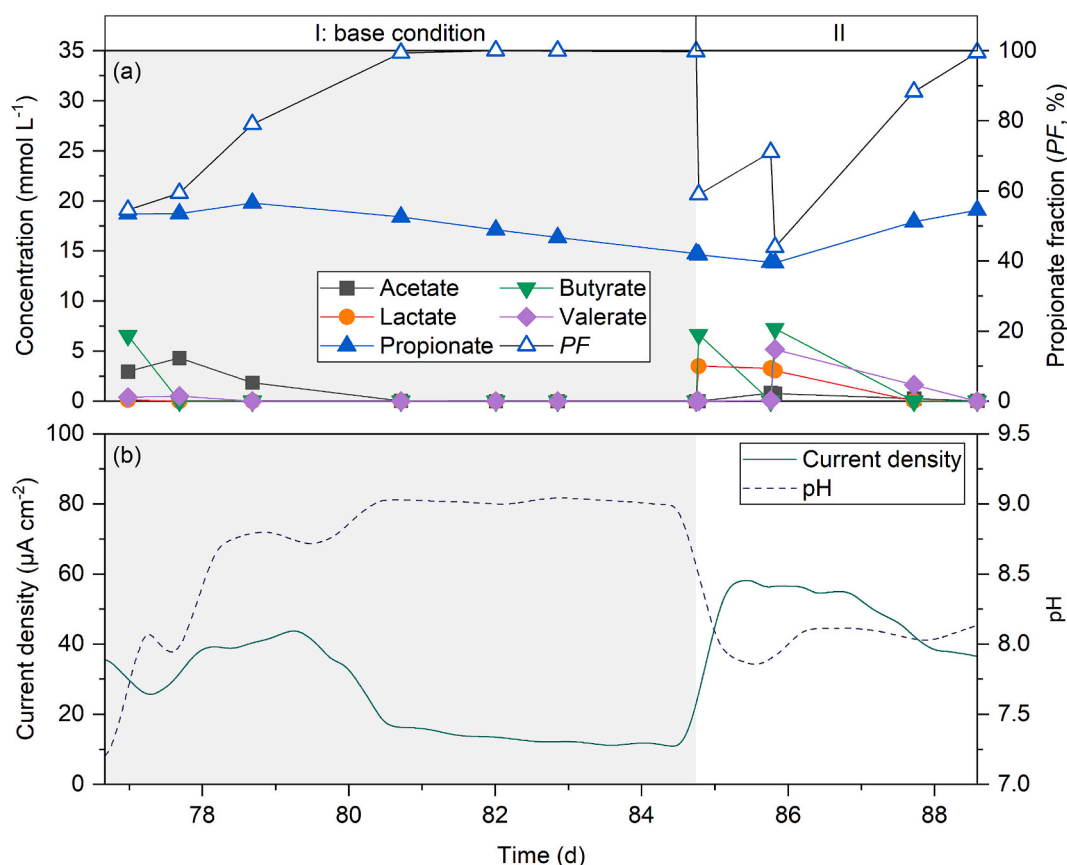


Fig. 4. (a) Development of SCFAs and propionate fraction among SCFAs (PF) in the final batch phase of Run 2 under alkaline conditions, and (b) corresponding pH values and current generation. At the onset of Phase I, a portion of the reactor medium was replaced with fresh hydrolysate and the pH was adjusted to above 8. During Phase II, butyrate, lactate and valerate were added to the reactor to verify that the microbial consortium was still active.

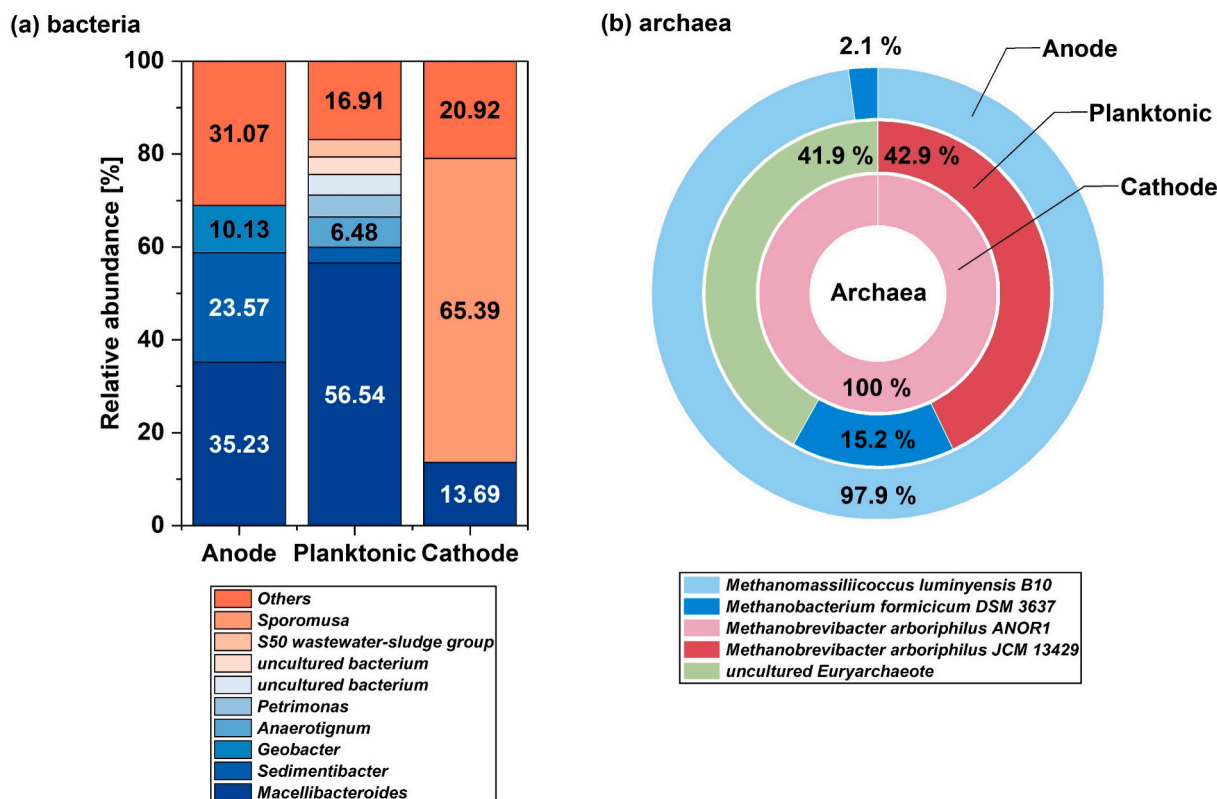


Fig. 5. Microbial communities of anodic and cathodic biofilms as well as of the planktonic phase at the end of Run 2. Taxonomic profiles of (a) bacteria were set at the genus level, whereas taxonomic profiles of (b) archaea were set at the species level. “Others” represent the sum of relative abundances of microorganisms with values lower than 3%.

3.2.3. Microbial community composition

To identify the composition of the microbial community responsible for propionate purification and gaseous biofuel generation in the reactor, at the end of Run 2, biofilm samples from the anodic and cathodic surfaces, as well as planktonic cells from the liquid phase, were collected and analyzed. The distributions of bacteria at the genus level and archaea at the species level are presented in Fig. 5. Both domains were investigated separately, and therefore the ratio of archaea and bacteria was not determined. It should additionally be noted that some bacterial taxa could only be confidently classified to the genus level. Possible metabolic pathways were inferred based on the reported metabolic traits of representative or well-characterized species from the identified genera. These functional assignments are hypothetical and may not reflect the actual metabolic capabilities of the strains present in the reactor. Moreover, taxonomic abundance alone does not demonstrate that a specific taxon is responsible for a particular metabolic conversion.

Among the bacteria, *Macellibacteroides*, *Sedimentibacter* and *Geobacter* were the predominant genera in the anodic biofilm, with relative abundances of 35.23%, 23.57%, and 10.13%, respectively (Fig. 5(a)). *Macellibacteroides* has been considered as strictly anaerobic, fermentative bacterium capable of converting carbohydrates into SCFAs [50,51]. Species of the genus *Geobacter* are well known for their capability of exoelectrogenesis [52]. For instance, *G. sulfurreducens* can oxidize acetate, lactate, formate and H_2 using an anode as the terminal electron acceptor, whereas *G. toluenoxidans* has been reported to oxidize acetate, propionate and butyrate by reducing ferrihydrite or ferric citrate [53,54]. The range of electron donors utilized varies considerably among *Geobacter* species and is considered an important determinant of their ecological niche [55]. Furthermore, *Geobacter* species have been reported to engage in cooperative interactions with fermentative microorganisms through interspecies electron transfer, thereby influencing

the metabolic pathways [56]. Therefore, the coexistence of *Macellibacteroides* with exoelectrogens like *Geobacter*, which accounted for 10% of the anodic biofilm, may indicate a potential commensal relationship between the two groups.

The cathode surface was predominantly colonized by the *Sporomusa* genus, which accounted for 65.39% of the bacterial community. *Sporomusa*, identified predominantly in the cathodic biofilm and only to a minor extent in the planktonic phase, is capable of autotrophically metabolizing H_2 and CO_2 to generate acetate [57]. Its presence alongside *Geobacter* in the anodic biofilm is consistent with the syntrophic relationship proposed by Elreedy et al. [23], in which butyrate oxidation by *Geobacter* generates acetate and H_2 , while *Sporomusa* consumes the produced H_2 .

In the planktonic phase, *Macellibacteroides* and *Anaerotignum* were the dominating genera, with *Macellibacteroides* accounting for more than 50% of the bacterial community and *Anaerotignum* representing 6.5%. Lactate produced from *Macellibacteroides fermentans* could serve as a substrate for propionate production by *Anaerotignum* [58], which convert D-lactate into propionate as the main SCFA. *Paludibacter*, which has been reported as a propionate producer in electrofermentation [59], was detected in Run 2, although at a relatively low abundance (2.0% of the bacterial community). Notably, classic propionate producers such as *Propionibacterium acidipropionici*, *P. shermanii* and *P. freudenreichii* [39] were not detected in the reactor. A similar observation was reported by Huang et al. [60], who found that obligate propionate producers were neither dominant nor detectable in the fermentation inoculated with sludge. The findings suggest that propionate formation may result from metabolic cooperation between the bacteria at different trophic levels, in which interconnected metabolic pathways convert complex substrates, such as amino acids and carbohydrates, into propionate. These propionate producers were located in the planktonic phase rather than on the electrodes. The electrode-associated organisms, *Geobacter* at the

anode and *Sporomusa* at the cathode, oxidize butyrate and acetate or form acetate, respectively, and do not produce propionate, and the only taxon linked to electrofermentation, *Paludibacter*, was a minor component present solely in Run 2. Propionate enrichment therefore reflects indirect electrode effects like the selective anodic removal of butyrate (and partly acetate) and the maintenance of a reducing bulk environment, rather than a direct electrode-driven regulation of the intracellular NAD^+/NADH ratio of the propionate-forming population.

The archaeal community was also characterized to account for the methanogenesis happening in the reactor, as shown in Fig. 5(b). *Methanomassiliicoccus luminyensis*, which dominated the archaeal portion of the community on the anode, has been reported to use H_2 as an electron donor but cannot reduce CO_2 ; methane production occurs via the reduction of methylated compounds, such as methanol [61,62]. It cannot be classified into the typical hydrogenotrophic or methylotrophic methanogenesis groups, because the reduction of methyl compounds is H_2 -dependent [63]. On the cathode and in the planktonic phase, *Methanobrevibacter arboriphilus* species, which are hydrogenotrophic methanogens, were detected [64]. The presence of both H_2 -dependent methanogens, *M. luminyensis* and *M. arboriphilus*, in the biofilm and planktonic phases provides a plausible explanation for the low hydrogen fraction in the produced gases in both runs. The detection of archaea in both anodic and cathodic biofilms suggests that methane generation occurs at least partly via immobilized biomass within the reactor. The coexistence of electroactive, fermentative, acetogenic and methanogenic microorganisms in the reactor highlights the complexity of the microbial consortium originating from the environmental inoculum. SCFA conversion may therefore involve complementary activities of electroactive bacteria and microorganisms associated with anaerobic digestion. However, because an open-circuit control was not performed under comparable conditions, the respective contributions of electroactive and other anaerobic pathways to the observed SCFA conversion cannot be quantitatively distinguished in the present study.

3.2.4. Comparative assessment of both runs and literature studies

The performance of the RDBER was evaluated for the continuous operation of both runs, and the corresponding performance metrics are presented in Fig. 6. These metrics include propionate purification, MEC performance and gaseous fuel generation. As discussed previously, the initial continuous phases in both runs included the transition from batch to continuous operation and from a synthetic medium to a hydrolysate. Obviously, the system had not yet reached a stable state in the first HRT testing phases of both runs, represented by substantial fluctuations and high coefficients of variation in propionate fraction (*PF*), its enrichment factor (*PEF*) and the net propionate production rate ($\overline{\text{NPPR}}$), as shown in Fig. 6(a)–(f). Therefore, these phases (indicated by the grey background in Fig. 6) were excluded from the following analyses and comparisons between the two runs. It should be noted that the effects observed across the HRT phases should not be interpreted as isolated effects of HRT, as the HRTs were investigated sequentially, which might imply changes in microbial community composition and biofilm development in general (coverage, thickness, etc.). Additionally, the inoculum used in Run 2 underwent a longer enrichment period and micro-aeration events (HRT of 4 d) may have long-term effects. Furthermore, changes in HRT may have affected the reactor pH, as observed in Run 1, introducing further interactions between operating parameters.

Following stabilization of the pH conditions in Run 2, the *PF* at the HRT of 4 days increased from $46.1 \pm 7.8\%$ in Run 1 to $61.8 \pm 10.1\%$ in Run 2, while the *PEF* remained relatively stable at 1.8 ± 0.5 and 2.0 ± 0.1 , respectively. These results indicate an improvement in propionate purification performance under stable neutral pH conditions. As presented in Fig. 6(g) and (h), the large difference in current density at an HRT of 4 days ($93.5 \pm 11.0 \mu\text{A cm}^{-2}$ in Run 1 vs $18.6 \pm 2.6 \mu\text{A cm}^{-2}$ in Run 2) may be attributed to the occurrence of hydrogen shuttling within the exoelectrogenic community in the reactor [27], whereby hydrogen

served as an additional electron donor for the anodic electron transfer, thereby enhancing the measured current. The difference is unlikely to be associated with variations in methanogenic activity, as both runs exhibited comparable methane fractions ($59.0 \pm 2.6\%$ in Run 1 and $62.7 \pm 0.6\%$ in Run 2) and similar gas production rates (0.45 and $0.41 \text{ L}_{\text{gas}} \text{ L}_{\text{liquid}}^{-1} \text{ d}^{-1}$ for Run 1 and Run 2, respectively) (Fig. 6(i)–(l)).

With pH stabilization in Run 2, operation at an HRT of 8 days achieved the highest propionate purification, with an average propionate fraction of $91.8 \pm 3.0\%$ and a maximum of 95.5% (Fig. 6(b)), which is beneficial for downstream separation and purification. However, in comparison to the HRT of 4 d, this improvement in purification efficiency was accompanied by a decline in volumetric output rate with a *PVOR* of $3.5 \text{ mmol L}^{-1} \text{ d}^{-1}$ due to the lower feeding rate (Fig. 6(f)). Thus, this trade-off has to be considered when choosing the HRT. The general limitation of the reactor performance is probably linked to suboptimal hydrodynamic conditions and incomplete utilization of the electrode surface by the biofilm (see SM). Uneven biofilm distribution was observed both along the anode stack and across the radial direction, with less biofilm developing in the middle of the anode stack and near the disc centers. Limited substrate supply and/or product removal within the inter-anode gaps may have resulted from the co-rotation of adjacent anode discs, thereby restricting biofilm growth and overall system performance. Therefore, optimization of hydrodynamic conditions within the reactor may further increase biofilm coverage on the anode surfaces, thereby enhancing the volumetric productivity.

For comparison with findings from literature, Table 3 compiles reported propionate fractions among SCFAs from studies targeting the production of propionate-rich streams (units used for SCFA quantification vary). It is noteworthy that (bio)electrochemical approaches generally achieved higher propionate fractions compared with other treatment strategies. Although the propionate fraction (on SCFAs basis) was not explicitly reported by Schmidt et al. [22], it can be inferred to have exceeded 90%, as acetate and butyrate were almost completely depleted. In that study, a similar concept was employed, involving the biological selective removal of non-propionate SCFAs to achieve propionate dominance. However, the process relied on inoculum with five pure cultures, which may be difficult to implement and maintain under more realistic conditions. Zeng et al. [65] applied a short-term electrochemical pretreatment (EPT) to sludge prior to anaerobic digestion and reported that propionate accounted for 89% of SCFAs with carbon-based electrodes. This behavior was attributed to the inhibition of methanogenesis and the enrichment of propionate-producing microorganisms. This strategy offers an alternative approach for generating propionate-rich streams from waste, but its long-term operational stability has yet to be fully evaluated. Another strategy for producing propionate-rich streams involves separation of fermentation stages, where an initial lactate-rich fermentation liquid is subsequently used as a substrate for propionate production. The highest propionate fraction of 69.9% achieved via this approach was reported by Li et al. [66]. Overall, the process developed in our study demonstrates high efficiency in the purification of propionate from SCFA mixtures, which was achieved at a reactor scale increased by a factor of between 3.1 and 78.3.

3.3. Preliminary considerations on process economics and environmental implications

The proposed process is not intended to replace established SCFAs recovery technologies but rather to function as an upstream selective purification step. By selectively removing the co-existing SCFAs with similar properties from the mixture, the process generates a propionate-dominant stream that can subsequently be refined using conventional separation technologies. From a preliminary process-economic perspective, two aspects are particularly relevant: (i) the relative market value of purified single acids versus mixed SCFA streams, and (ii) the economic and environmental implications of introducing an upstream

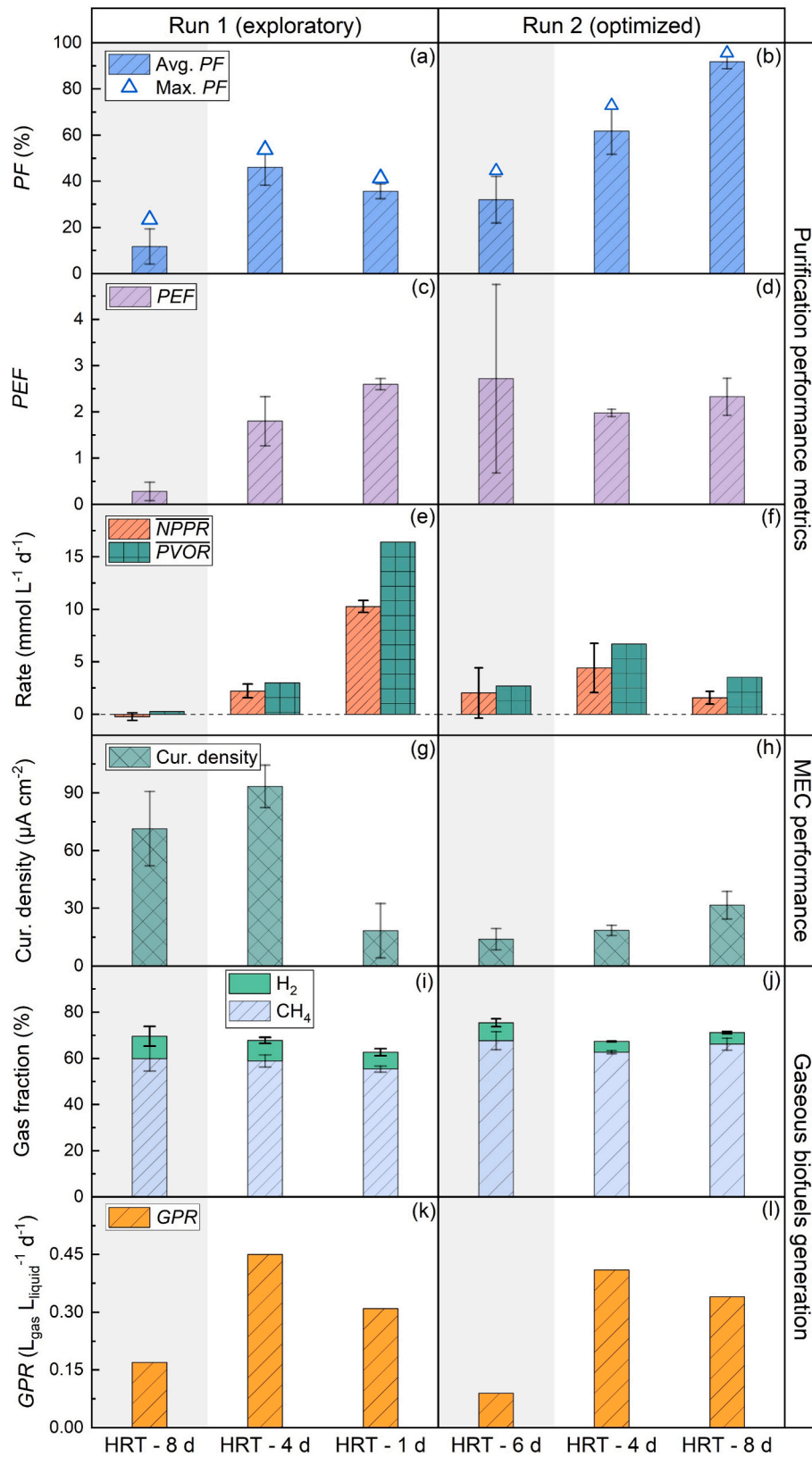


Fig. 6. System performance metrics of Run 1 and 2 for different hydraulic retention times (HRT) during continuous operation. (a, b) Average and maximum propionate fraction (PF); (c, d) propionate enrichment factor (PEF); (e, f) average net propionate production rate ($\overline{NPPR}$) and average propionate volumetric output rate ($\overline{PVOR}$); (g, h) current density; (i, j) H₂ and CH₄ fractions in the generated gas; and (k, l) gas production rate (GPR).

Table 3
Comparison of the propionate (prop) fraction among SCFAs reported in this study and previous publications.

Reactor	Working volume	Key process feature	Inoculum	Substrate	Temp. (°C)	pH	Operational mode	Max. prop fraction	Prop conc.	Reference
Anaerobic sequencing batch reactor	1.6 L	960 mg L ⁻¹ FeCl ₃	Anaerobic digestion sludge	Synthetic WW (starch as sole carbon)	37	8.0	Continuous (HRT-24 h)	70.74% (g COD L ⁻¹ ; SCFAs)	4.6 g L ⁻¹	[8]
Fermentation reactor	1.8 L	Thermally pretreated dog food	Soft goat cheese	Vegan Dog food	30	6.0	Batch	55% (g L ⁻¹ ; SCFAs)	26.5 g L ⁻¹	[9]
Microbial fuel cell	270 mL	Two-chamber reactor	<i>Geobacter sulfurreducens</i> <i>Shewanella oneidensis</i> <i>Escherichia coli</i> <i>Enterococcus faecium</i> <i>Shewanella putrefaciens</i>	Hydrolysate	–	7.0	Fed-batch	> 90% (estimated, mmol L ⁻¹ or g COD L ⁻¹ ; SCFAs)	1.8 g L ⁻¹	[22]
Anaerobic tank	2 L	2 stage process (food waste-lactate-propionate)	Twelve lactate-producing strains	Food waste	35	4.0	Semi-continuous	45.26 ± 2.23% (g COD L ⁻¹ ; SCFAs)	–	[33]
			Bovine rumen	Lactate-rich liquid from 1st stage	35	5.0	Semi-continuous			
	1.2 L	2 stage process (food waste-lactate-propionate)	Pig intestines microbiome anaerobic sludge	Food waste	21 ± 1	8.2	Batch	68.4% (g COD L ⁻¹ ; SCFAs)	4.72 g L ⁻¹	[67]
			<i>Propionibacterium acidipropionici</i>	Lactate-rich liquid from 1st stage	30 ± 1	7.0	Batch			
	3.0 L	2 stage process (food waste-lactate-propionate)	Sludge	Food waste	50 °C for 4 h and 35 °C for 48 h	8.2 ± 0.4	Batch	69.9% (g COD L ⁻¹ ; SCFAs)	7.1 ± 0.3 g L ⁻¹	[66]
				Lactate-rich liquid from 1st stage	30	7.0 ± 0.5	Batch			
	1.1 L		<i>Propionibacterium acidipropionici</i>	Lactate-rich liquid from 1st stage	30	7.0 ± 0.5	Batch			
Serum bottles	120 mL	Electrochemical pretreatment of WWTP sludge (12 V for 30 min)	Anaerobic digestion sludge	Waste-activated sludge from WWTP	35 ± 1	6.7	Batch	89% (based on the assumption of mmol L ⁻¹ ; SCFAs)	–	[65]
RDBER	9.4 L	Selective removal of SCFAs	<i>Geobacter sulfurreducens</i> Butyrate-oxidizing consortium	Diluted hydrolysate	30	7.0	Continuous (HRT – 8 d)	95.5% (mmol L ⁻¹ ; SCFAs) 97.3% (g COD L ⁻¹ ; SCFAs)	2.1 g L ⁻¹	This study

WW – wastewater, WWTP – wastewater treatment plant, TOC – total organic carbon.

biological purification step.

As reported in an EU project [68], SCFA mixtures in aqueous solution are valued at approximately 500–600 € ton⁻¹ (corresponding to a 5–8% VFA solution), whereas acetic acid, propionic acid, and butyric acid solutions are valued at approximately 600–800, 1800–2000, and 1200–1400 € ton⁻¹, respectively. Although the expected concentrations of these solutions are not explicitly specified, these values indicate a higher market value for individual acids compared with mixed streams. In practice, the mixtures of SCFAs are predominantly used as bulk carbon sources for enhanced nutrient removal in wastewater treatment, as feedstocks for biodiesel-related processes, as well as for poly-hydroxyalkanoates (PHA) synthesis [69–71], rather than as direct commercial commodities. In contrast, propionic acid is a well-established platform chemical with a broader range of high-value applications, including its use as an antimicrobial agent in food preservation, an anti-inflammatory agent in pharmaceuticals applications, and an active ingredient to produce herbicides [6].

Thanahiranya et al. [72] reported that propionic acid recovery from glycerol fermentation requires reactive extraction followed by multiple distillation stages, indicating substantial downstream processing demands. In mixed SCFA streams, the additional requirement to selectively separate propionic acid from coexisting acids further increases separation complexity and potentially the associated energy consumption. MECs require an external voltage supply, typically in the range of 0.2 to 0.8 V [73]. In this study, the MEC was primarily used as a reactor platform rather than a hydrogen-production system. Owing to the low current density resulting from concurrent methanogenesis (e.g., $31.7 \pm 7.2 \mu\text{A cm}^{-2}$ at the HRT of 8 days in Run 2), the electrical power demand for the MEC process was estimated to be $5.8 \times 10^{-3} \text{ kWh d}^{-1}$ at an assumed applied voltage of 0.8 V and a current of 0.3 A. (Fig. 6(g, h)). In addition to the electrochemical energy requirement, the mechanical energy demand of Run 2 mainly comprises four components (excluding the water-heating jacket): energy for disc rotation, liquid recirculation, vessel mixing and liquid feeding. The mixing vessel was used specifically for the micro-aeration experiment and is not required for the proposed process. The rotor for the discs was operated at 12 V and 0.2–0.3 A, corresponding to $5.8\text{--}8.6 \times 10^{-2} \text{ kWh d}^{-1}$. The recirculation gear pump (ISMATEC, ISM918A) had a rated maximum power consumption of 150 W but was operated at only 500 mL min^{-1} (< 10% of its maximum operating speed), while the feeding pump (ISMATEC ISM829) had a rated maximum power consumption of 20 W and was operated between 6 and 12 rpm (maximum available speed: 99 rpm). Therefore, the actual power consumption of both pumps was expected to be lower than their rated maximum values. As the pump power was not directly measured, their actual energy demand could not be reliably quantified. Overall, while the electrochemical energy demand was relatively low, mechanical energy consumption may contribute substantially to the overall energy demand of the process and should be directly quantified in future studies.

Furthermore, the hydrolysate was diluted tenfold and autoclaved to facilitate practical long-term hydrolysate supply and storage while mitigating potential inhibition associated with high SCFA concentrations. However, these pretreatment steps may introduce additional energy requirements in both the proposed purification process and the downstream processing. Autoclaving also removed the microbial population from the hydrolysate which may otherwise compete with or influence the working consortium. In addition, pH adjustment was not included in the present energy assessment. It also does not account for downstream concentration and recovery of propionate, which may involve additional energy and chemical requirements depending on the selected recovery strategy. Further evaluation using less-diluted, non-autoclaved hydrolysate and incorporating downstream processing is therefore needed to assess the process under more energy-efficient and practical conditions.

In addition to the economic considerations, the proposed process also offers environmental benefits. The degradation of non-propionate

SCFAs and related compounds resulted in the production of gaseous biofuels rather than complete mineralization to CO₂. Gaseous biofuels were generated at a volumetric rate of approximately $0.4 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ and the methane content was within the range typically reported for anaerobic digestion and was comparatively high among reported biogas compositions [74]. Besides, the biogas contained 5–10% hydrogen, which further increased its energy value by enhancing the overall energy content. These preliminary assessments indicate a potential techno-economic relevance of the proposed concept, while a detailed evaluation will be required in further scale-up studies.

4. Conclusions

Propionate purification from SCFA mixtures in dark fermentation hydrolysate was successfully enhanced using a bioelectrochemical approach. Both HRT and pH significantly influenced propionate purification efficiency and process stability. The process enables selective removal of non-propionate SCFAs and in-situ propionate formation, leading to a significant increase of the propionate fraction in the treated effluent. Concurrent production of gaseous biofuels may provide an additional energy benefit to the process. These findings confirm the feasibility of MECs inoculated with *G. sulfurreducens* and a butyrate-oxidizing consortium as an effective propionate purification step prior to downstream separation.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used ChatGPT and ProWritingAid in order to perform language editing to increase readability of the article. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRedit authorship contribution statement

Zhizhao Xiao: Investigation, Conceptualization, Methodology, Visualization, Writing – original draft. **Magdalena Harz Fresno:** Investigation. **Johannes Eberhard Reiner:** Investigation, Writing – review & editing. **Ahmed Elreedy:** Methodology, Resources, Writing – review & editing. **Johannes Gescher:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Harald Horn:** Conceptualization, Funding acquisition, Writing – review & editing. **Andrea Hille-Reichel:** Conceptualization, Methodology, Writing – review & editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seppur.2026.140280>.

Data availability

Data will be made available on request.

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