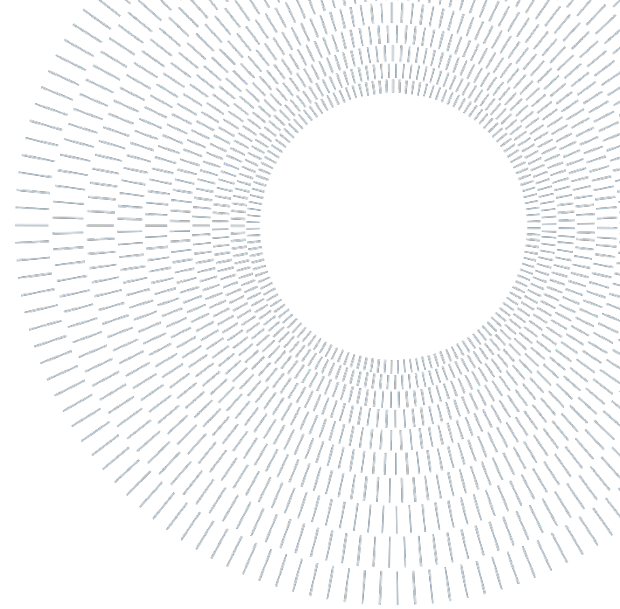




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EXECUTIVE SUMMARY OF THE THESIS

Upscaling Strategy for Bio-based Volatile Fatty Acids from Industrial Wastes and Residual Streams

TESI MAGISTRALE IN CHEMICAL ENGINEERING – INGEGNERIA CHIMICA

AUTHOR: MARGHERITA BECHI

ADVISOR: ALBERTO CUOCI

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This research project was conducted in collaboration with the Department of Industrial Biotechnology at the KTH Royal Institute of Technology in Stockholm. I had the honour of working with the Bioconversion Group under the supervision of Zeynep Cetecioglu Gurol and Isaac Owusu-Agyeman, who guided me through the fascinating world of waste valorisation.

1. Introduction

The excessive use of resources has boosted the use of fossil raw materials to meet growing energy demand. Transitioning towards more sustainable production routes is the only way to face the escalating worldwide trend of waste generation, targeting UN Sustainable Development Goals 6, 7 and 11 and the global temperature reduction of 1.5°C, as outlined in the Paris Agreement in 2016. Biorefinery strategies can convert carbon-rich industrial effluents into high-value goods, transforming wastewater treatment plants (WWTPs) from pollution control facilities into resource recovery centres, and fostering a bio-based circular economy [1].

Volatile fatty acids (VFAs) are versatile chemical building blocks that find applications in the pharmaceutical, food, and chemical industries. Traditionally they are derived from petroleum-based sources via chemical routes. The most promising alternative pathway from industrial wastes and residual streams is tailored anaerobic

digestion (AD) through Microbial Mixed Culture (MMC). VFAs are indeed intermediates of the conventional AD process exploited for biogas production. To shift towards VFAs, the operating parameters of the four metabolic phases (i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis) must be tuned, ultimately arresting the final step of methanogenesis [2]. Mixed culture fermentation is a cost-effective and flexible alternative to single-strain cultures able to bridge waste streams and bio-based VFA production. The inoculum of these microbial consortia consists of the sludges extracted from WWTPs, directly fed into the anaerobic digestion system without any need for sterile conditions. Among different industrial effluents, acidic whey (a co-product from the manufacture of acid-coagulated cheeses, like ricotta and Greek yoghurt) and vinasse (a by-product from the yeast and ethanol industry) are the most attractive, thanks to their high carbon content (chemical oxygen demand COD > 4 g/L), good biodegradability, and high production volumes.

MMC-tailored AD towards bio-based VFAs faces challenges like low yields and productivity, lack of cost-effective separation methods, and on top of all, scalability [1]. The effects of design parameters (i.e., pH, retention time, substrate, inoculum, and bioreactor configuration) on VFA production and composition are widely reported. However, only a few research studies investigated the transition from batch to semi-continuous long-term setups.

This project targeted increasing efficiency, suggesting an upscaling strategy to fill the current literature gap towards a full-scale implementation of bio-based VFA. Firstly, a batch experiment was conducted to evaluate the potential of three different industrial waste and residual streams i.e., acidic whey, lactose-free (LF) acidic whey, and vinasse under initial alkaline and acidic environments. Then, the most effective operating conditions were transitioned to an anaerobic sequencing batch reactor (AnSBR) setup, applying different organic loading rates (OLRs).

2. Materials and methods

2.1. Substrates and Inoculums

Acid whey and lactose-free (LF) acid whey were collected from Skånemejerier AB, Malmö, one of the largest dairy companies in Sweden. Vinasse was received from Jästbolaget Aktiebolag AB, Sollentuna, Sweden after being subjected to an evaporation process. All the relevant properties are summarised in Table 2.

Parameters	Vinasse	Acidic Whey	LF Acidic Whey
TS (g/L)	730.9 ± 20.5	54.5 ± 0.4	54.3 ± 0.7
VS (g/L)	580.0 ± 21.5	46.9 ± 0.4	46.8 ± 0.7
COD _{Total} (g/L)	509.4 ± 0.2	72.4 ± 2.5	67.4 ± 1.9
COD _{Soluble} (g/L)	493.2 ± 1.2	63.5 ± 0.4	61.5 ± 0.3
Total P (g/L)	0.81 ± 0.15	0.78 ± 0.02	0.75 ± 0.01
PO ₄ ⁽³⁻⁾ -P (mg/L)	25.4 ± 0.7	191.2 ± 3.1	196.9 ± 0.8
Total N (g/L)	54.3 ± 2.5	0.52 ± 0.00	0.56 ± 0.01
NH ₄ ⁽⁺⁾ -N (g/L)	0.38 ± 0.02	0.09 ± 0.00	0.13 ± 0.00
VFA (gCOD _{VFA} /L)	0.23 ± 0.08	0.25 ± 0.12	0.25 ± 0.08
Density ρ (g/mL)	1.18 ± 0.02	1.00 ± 0.00	1.02 ± 0.01
pH	7.0 ± 0.0	4.2 ± 0.0	4.3 ± 0.0

Table 2: Characterisation of substrates.

Two slurry-digested sludges were collected from a full-scale anaerobic digester, at Henriksdal Wastewater Treatment Plant, Stockholm, Sweden.

Sludge 1 (Table 1) was used during the anaerobic batch tests for the acidogenic fermentation of vinasse, acidic whey, and LF acidic whey. Sludge 2 (Table 1) was inoculated during the AnSBR setup with LF acidic whey. Before the experiments, substrates and inoculums were stored at 4°C for two weeks without any further pre-treatment.

Parameters	Sludge 1	Sludge 2
TS (g/L)	24.5 ± 1.3	22.9 ± 1.6
VS (g/L)	16.6 ± 0.5	14.0 ± 0.3
COD _{Total} (g/L)	22.5 ± 0.9	20.0 ± 1.4
COD _{Soluble} (g/L)	-	13.2 ± 0.9
Total P (g/L)	26.1 ± 2.0	30.6 ± 0.2
PO ₄ ⁽³⁻⁾ -P (mg/L)	0.6 ± 0.0	0.4 ± 0.0
Total N (g/L)	0.620 ± 0.01	0.578 ± 0.02
NH ₄ ⁽⁺⁾ -N (g/L)	0.45 ± 0.08	< 2
VFA (gCOD _{VFA} /L)	1.8 ± 0.1	1.6 ± 0.06
Density ρ (g/mL)	1.1 ± 0.01	1.1 ± 0.03
Alkalinity (mgCaCO ₃ /L)	5000.0	3600.0
pH	8.1 ± 0.0	7.3 ± 0.0

Table 1: Characterisation of inoculums.

2.2. Experimental Setups

2.2.1. Anaerobic Batch Tests

Anaerobic batch experiments were conducted using an automatic methane potential test system (AMPTS II, Bioprocess Control, Sweden). The equipment consisted of glass bottles with a working volume of 450 mL placed in a water bath, connected to a CO₂-capturing unit (3 M NaOH with 0.4% Thymolphthalein pH indicator) and a biogas measuring device.

Acidic whey, LF acidic whey, and vinasse's acidogenic fermentations were tested under an initial pH of 5 and 9. Each reactor was loaded with an amount of substrate equal to a total chemical oxygen demand (COD) concentration of 16 g/L, whilst the amount of sludge (Sludge 1, Table 1) was set at a volatile solid (VS) content of 8 g/L. This to achieve the optimum substrate/inoculum ratio for VFA production of 2 g COD/g VS [3]. 10 mL of a trace element solution (2 mg/L FeCl₂·4H₂O, 2 mg/L CoCl₂·6H₂O, 0.32 mg/L MnCl₂, 0.024 mg/L CuCl₂, 0.05 mg/L ZnCl₂, 0.05 mg/L H₃BO₃, 0.09 mg/L (NH₄)₂MoO₇·24H₂O, 0.068 mg/L Na₂SeO₃, 0.05 mg/L NiCl₂·6H₂O, 1 mg/L EDTA, 0.001 mL HCl (36%)) were diluted into 1 L of tap water left in a fume hood overnight for chlorine removal. The as-created solution was used to dilute the reactors' content to the desired COD concentrations.

At the beginning of the setup, the pH was adjusted to 5 and 9 by using 1 M HCl and 1 M NaOH, 5 and 9, respectively. Each reactor was flushed with nitrogen for 10 min after filling to ensure anaerobic conditions. Experiments were conducted in triplicates, with an overall amount of eighteen glass bottles. The reactors were operated for 15 days with 120 rpm mixing at 35 °C.

2.2.2. Anaerobic Sequencing Batch Test

Two anaerobic sequencing batch reactors (AnSBRs) from AMPTS® II Light units were used for the upscaled semi-continuous study. They had a 2000 mL total volume and 1400 mL active volume and were run by cycling through a series of four phases in a single reaction vessel. One cycle lasted 24 hours, with 5 minutes dedicated to feeding, 20 hours to reacting, 3 hours to settling, and 5 minutes to decant. The discharge and the feeding for each cycle were carried out through two peristaltic pumps and accounted for about 30% of the active volume (i.e., 400 mL) [4]. The reactors were flushed with nitrogen for 10 minutes at the beginning of the set-up to ensure anaerobic conditions. The AnSBRs were adjusted in a water bath to control the temperature to 35°C and were equipped with an automatic stirring system fixed at 20 rpm.

Based on the previous results, LF acidic whey was selected as the substrate. The hydraulic retention time (HRT) was fixed to 3.5 days thanks to the good VFA accumulation potential the dairy effluent showed within the first days of the previous batch experiment. For longer reaction operations, acidic pH was proven to enhance VFA production from the fermentation of dairy wastewater. Therefore, the pH was adjusted and controlled to 5 by dosing 1 M of NaOH. Anaerobic digested sludge (Sludge 2, Table 1) was used as inoculum with a concentration of 9.3 g VS/L in each reactor. The operation of the ASBRs consisted of a sequence of three different organic loading rates (OLR) and lasted 41 days. The reactors were first run for two weeks with an OLR of 2.80 g COD /L/day with a ratio of food to microorganism (F/M) equal to 0.3 g COD/g VS. Then in a stepwise manner, the OLR was increased to 4.67 g COD/L/day reaching a 0.5 F/M ratio. Lastly, feed concentration was adjusted to 23 g COD/L, corresponding to an OLR of 6.53 g COD/L/day and a 0.7 F/M ratio. Based on the COD concentrations in the influent and effluent, it was ensured that steady-state conditions were reached during each phase.

2.3. Analytical Methods

As for the anaerobic batch test, soluble chemical oxygen demand (COD), volatile fatty acids (VFAs), and biomethane production were measured every two days. The pH was not controlled but monitored daily. During the AnSBR setup instead, total and volatile solids (TS and VS), total suspended and volatile suspended solids (TSS and VSS), soluble COD, and VFA production were measured in the effluents at least twice per week. Reactors' pH was monitored daily and controlled. VFA concentration and composition in the samples were analysed by gas chromatography (Intuvo 9000 GC System, Agilent) equipped with a Flame Ionization Detector (FID) and a capillary column (DB-WAX Ultra Inert, 30 m x 250 µm x 0.25 µm, Agilent 122-7032UI), where helium was used as the carrier gas at a flow rate of 2 mL/min. Each sample was first centrifuged for 10 minutes at 4300 rpm and then filtered through 0.2 mm polypropylene syringe filters. Before the GC analysis, a sample aliquot of 0.5 mL was acidified with 100 µL orthophosphoric acid (H₃PO₄ 25%) in a glass vial of 1.5 mL and stored in the fridge at 4°C.

The final VFA concentration was expressed in COD units through stoichiometric COD factors of 1.0667 for acetic acid, 1.512 for propionic acid, 1.813 for butyric acid and iso-butyric acid, 2.036 for valeric acid and iso-valeric acid, 2.207 for caproic acid and iso-caproic acid, and 2.34 for heptanoic acid [3]. The total and soluble CODs were spectrophotometrically analysed with cuvette test kits (LCK 514 Hach Lange, range 100-2000 mg/L). TS, VS, TSS, and VSS were measured according to the Standard Methods [5]. Hach cuvette tests were used to measure total phosphorus, orthophosphate, total nitrogen, and ammonium (APC350, range 2-20 mg/L PO₄-P; APC350 6-60 mg/L PO₄; LCK 338, range 20-100 mg/L TNb; LCK 303, range 2.5-60 mg/L). Lastly, the pH of samples was measured with a pH electrode (In Lab Expert Pro-ISM, Mettler Toledo) and a meter (Seven Compact S220, Mettler Toledo).

2.4. Computations

The VFA yield (Y_{VFA} , Equation 1) was calculated as the ratio of total VFAs produced in the effluent per unit of total COD concentration from the substrate fed to the reacting system.

$$Y_{VFA} = \frac{VFA\ prod}{COD_t\ Substrate} \quad (1)$$

where VFA_{prod} is the total VFA production (g COD-VFA/L) and $COD_{t\ Substrate}$ is the total COD concentration (g CODt/L) of the influent substrate. Moreover, for each sampling day, the degree of acidification (DA, Equation 2) was computed, by dividing the total VFAs (g COD-VFA/L) produced by the effluent soluble COD (g CODs/L):

$$DA = \frac{VFA_{prod}}{COD_s} \quad (2)$$

Lastly, for the batch sets, a carbon mass balance (Equation 3) was carried out to highlight the optimal operating conditions. The initial organic load of each reactor was assumed to be converted in part to cell tissue and in part to biomethane and VFAs, with the rest either remaining unconverted or forming undesired products (e.g., hydrogen, hydrogen sulphide, alcohols, and other non-organic carbon).

$$1 = Y_{VFA} + Y_{CH_4} + other\ products + COD_{unconv} \quad (3)$$

To make all these contributions sum up to one, biomethane production (BMP, expressed in g COD) was normalized with the total grams of COD from the industrial waste stream loaded in each reactor at the beginning of the setup, resulting in a methane yield (i.e., Y_{CH_4}).

3. Results and Discussion

3.1. Anaerobic Batch Tests

Anaerobic batch reactors were operated to reveal the effect of initial pH (5 and 9), type of substrate (acidic whey (AW), lactose-free (LF AW) acidic whey, and vinasse), and hydraulic retention time (HRT) on VFA production and composition over the 15-day trial. The results of the total VFA (g COD-VFA/L) are reported in Figure 1 along with the effluent VFA profile and the VFA production yield (g COD-VFA/g CODt fed). Figure 2 shows pH trends over the experiments. Lastly, Figure 3 displays the outcomes of the carbon mass balances.

3.1.1. Effects of substrate, pH, and HRT on VFA production and composition

The highest VFA concentration achieved with lactose-free (LF) acidic whey under initial pH 5 was 11.677 ± 0.177 g COD-VFA/L at day 15 (Figure 1b, on the left). The corresponding yield was 0.7 g COD-VFA/g CODt fed resulting in the overall best performance. Likewise, regular acidic whey's maximum results were 11.170 ± 0.248 g COD-

VFA/L and 0.66 g COD-VFA/g CODt, with pH of 5 and HRT of 15 days (Figure 1a, on the left). Under an acidic setup and for long HRT, the fermentation of the two dairy effluents performed better than at an initial pH of 9. However, with alkaline pH, the digestion evolved faster reaching a peak production of 11.479 ± 0.124 g COD/L on day 7 and 11.018 ± 0.344 g COD/L on day 9 for LF acidic whey and regular acidic whey, respectively (Figure 1a and Figure 1b, on the right). Accordingly, maximum yields were 0.69 and 0.66 g COD-VFA /g CODt. Moreover, the reactors operating in acidic environments kept producing VFAs until the end of the trial, with pH (Figure 2) remaining steady at values below 5. On the other hand, alkaline reactors' VFA production dropped from day 9 onward. Their pH, after a decrease in the first days down to 5, got stabilised to neutral /slightly alkaline values (i.e., 7.5), which are well within the range favourable for methanogenesis.

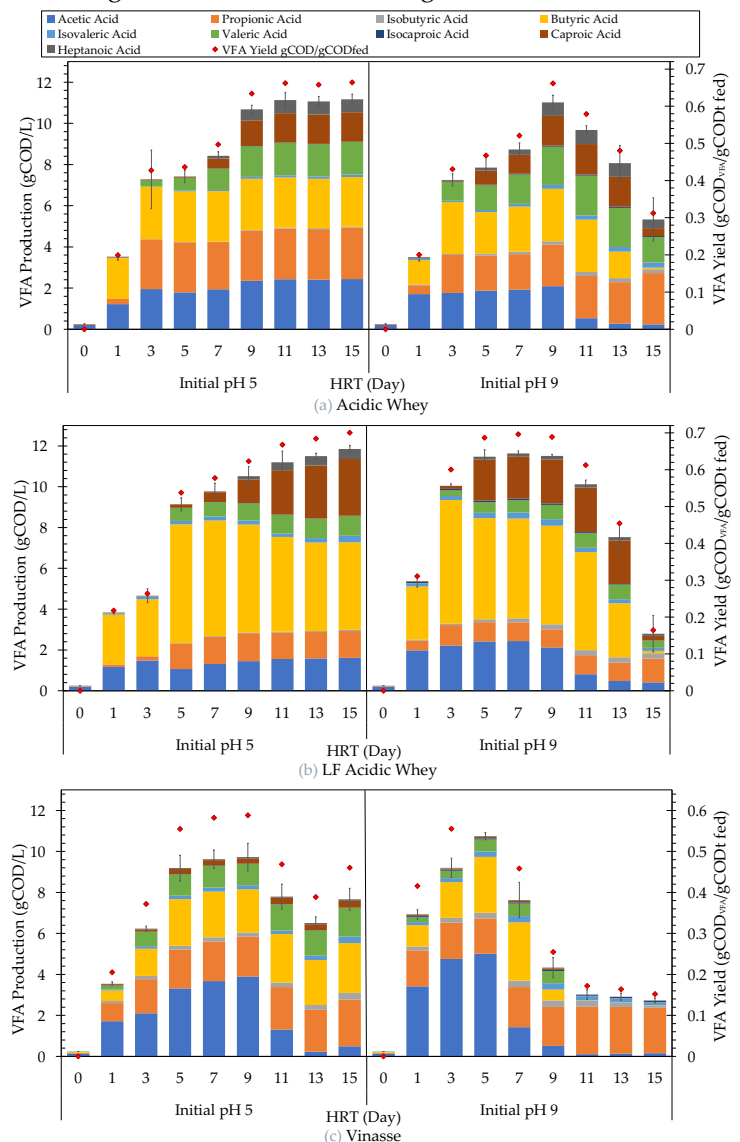


Figure 1: Anaerobic batch test results.

Differently, the peak VFA production from vinasse occurred under initial pH 9 at day 5. Despite achieving the best results for shorter HRT, the highest total organic acids' concentration was 10.761 ± 0.177 g COD-VFA/L, slightly lower compared to the other substrates. The corresponding yield was 0.65 g COD-VFA/g CODt (Figure 1c, on the right). Similarly to the dairy effluents, VFA production from vinasse alkaline batches sharply dropped in favour of methane and other metabolites after the 5th day, as proven by carbon mass balance (Figure 3c, on the right). In line with this, pH was steady at mild alkaline values (on average 7.91 ± 0.58). Under initial pH 5, instead, vinasse maximum VFA production was 9.712 ± 0.667 g COD-VFA/L and yielded 0.59 g COD-VFA/g CODt achieved after 9 days of HRT. The experiments proved vinasse's high buffer capacity and strong alkalinity: starting from pH 5, acidic batches steadily reached neutral values. Accordingly, total VFA production decreased from day 9 onward.

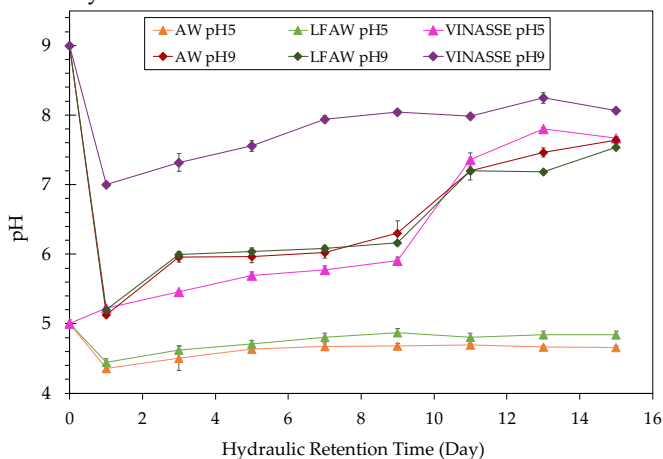


Figure 2: pH trends during batch tests.

Generally, alkaline environments have been reported to boost the rate-limiting step of the tailored-anaerobic digestion i.e., hydrolysis rate, acting as a pre-treatment step [6]. The resulting higher solubilisation of carbohydrates and proteins stimulates microbial growth, as well as acidogenic activity, ultimately enhancing VFA production. This holds for the LF acidic whey, whose VFA production under initial pH 9 was double the one registered under initial pH 5 at day 3 (9.936 ± 0.092 and 4.550 ± 0.335 g COD-VFA/L, respectively). Likewise, vinasse total VFA concentration showed much higher results in the alkaline sets compared to the acidic ones during the first days of the trial (i.e., for HRT < 5 days). However, the regular acidic whey behaved differently and reported VFA

concentrations below 8 g COD-VFA/L until day 5, regardless of the initial pH conditions. This trend could be the consequence of the demanding degradation of lactose. The presence of the disaccharide might have caused a larger concentration of soluble matter and organic overloading, ultimately resulting in a lag phase in the biological process. Following an adaptation stage, the production of VFAs increased significantly on day 9 (11.018 ± 0.344 g COD-VFA/L). Lagoa-Costa et al. reported the evolution of lactose consumption during cheese whey fermentation in AnSBR setups. The study revealed that VFA production began only after lactose was fully digested to lactate which then reached its maximum peak [7]. Similarly, Jankowska et al., for initial alkaline conditions, reported VFA between 0.09 and 0.14 g VFA/g CODs in the first days of the process. Only for longer HRT (i.e., from the 15th day), VFA production achieved a 5fold increase to 0.71 g VFA/g CODs [6].

Research indicates varying pH effects on substrates, with some favouring alkaline environments for VFA production, while others prefer neutral or acidic pH. As for this study, vinasse better performed under an initial pH of 9 and an average neutral reaction environment. In line with this, Eng et al. maximised VFA production from sugarcane vinasse at 39.6 °C and pH of 8.8 achieving a peak concentration of 2.980 g/L and a maximum VFA yield of 0.332 g COD-VFA/g CODt fed [8].

Comparative data on VFA production in cheese whey and sugarcane vinasse-fed batch systems are summarised in Table 3.

Substrate	V (L)	T (°C)	pH	HRT(d)	Yield	Ref.
Cheese Whey	0.06	35	Initial 5	10	0.49 gVFA/gCODs	[6]
LF Acidic Whey	0.500	35	Initial 5	15	0.70 gCOD _{VFA} /gCODt	This study
Acidic Whey	0.500	35	Initial 5	11	0.66 gCOD _{VFA} /gCODt	This study
Sugarcane Vinasse	0.500	39.6	8.8	7	0.332 gCOD _{VFA} /gCODt	[8]
Vinasse	0.500	35	Initial 9	5	0.65 gCOD _{VFA} /gCODt	This study

Table 3: Comparative results for batch trials.

The substrate type significantly impacted the final VFA composition, with acetic, propionic, butyric, valeric, and caproic acids being the dominant types

during the process, suggesting mixed-acid fermentation as the prevailing metabolic pathway. For the two wheys, VFA profiles were similar regardless of the pH. The regular acidic whey showed a homogenous distribution of acetic, propionic, and butyric acids with average concentrations of 1.60 ± 0.80 , 1.86 ± 0.82 , and 1.97 ± 0.84 g COD/L, respectively. Nonetheless, minor amounts of valeric (1.10 ± 0.68 g COD/L) and caproic (0.74 ± 0.64 g COD/L) acids were also detected (Figure 1a). Conversely, the LF acidic whey VFA profile displayed butyric acid as the dominant metabolite (35-64% of the total VFAs) with an average production of 3.89 ± 1.84 g COD/L (Figure 1b). For these two substrates, the influence of pH over the final VFA profile was minor. Nonetheless, alkaline batches favoured higher percentages of iso-butyric (0.7-8.6%), iso-valeric (0.97-7.23%), and iso-caproic (0.40-1.94%) acids compared to acidic setup. Lastly, HRT favoured chain elongation, boosting caproic acid production with peak concentrations of 1.43 and 2.78 g COD/L on day 15 for regular and LF acidic, respectively. On the other hand, the other acids showed constant values no matter the HRT. As for vinasse, VFA profiles (Figure 1c) display a stronger influence of reactors pH. Under initial acidic environments, the major acids were acetic (1.87 ± 1.42 g COD/L), propionic (1.64 ± 0.72 g COD/L), butyric (1.72 ± 0.89 g COD/L), and valeric (0.87 ± 0.49 g COD/L), with steady productions during the trial. Conversely, for the batches at initial pH 9, acetic and propionic acids prevailed with average concentrations of 1.74 ± 2.08 and 1.77 ± 0.69 g COD/L. Substrate composition is expected to influence the VFA profile, with more carbohydrates stimulating butyric acid production, whilst higher protein composition yielding more valeric acid [6]. In line with this, the effect of substrate type prevailed over pH and HRT during this study. Different starting compositions led to dissimilar metabolic pathways. Direct oxidation to butyric acid may have been the predominant route for the LF acidic whey. On the other hand, the presence of lactose in the regular acidic whey led to the production of lactic acid. This intermediate, being subjected in equal proportions to oxidation and reduction, was ultimately converted to propionic and butyric acid in similar amounts (18-33% and 16-35% of the total VFAs produced, respectively). Likewise, Jankowska et al., during a mixed culture batch fermentation with cheese whey at initial pH 5,

reported steady distributions of acetate (21-25%), propionate (18-20%), butyrate (15-20%), and valerate (16-20%) no matter the HRT. Vinasse's heterogenous VFA profile is slightly in disagreement with the literature. Eng et al., reported acetic and butyric as major VFAs under alkaline conditions, while iso-butyric and iso-valeric acids were favoured by alkaline pH [8]. Moreover, all the substrates, when the pH reached neutral/slightly alkaline values, registered a drop in acetate, suggesting its conversion to long-chain fatty acids, alcohols, hydrogen, and biogas, as confirmed by the carbon mass balance (Figure 3).

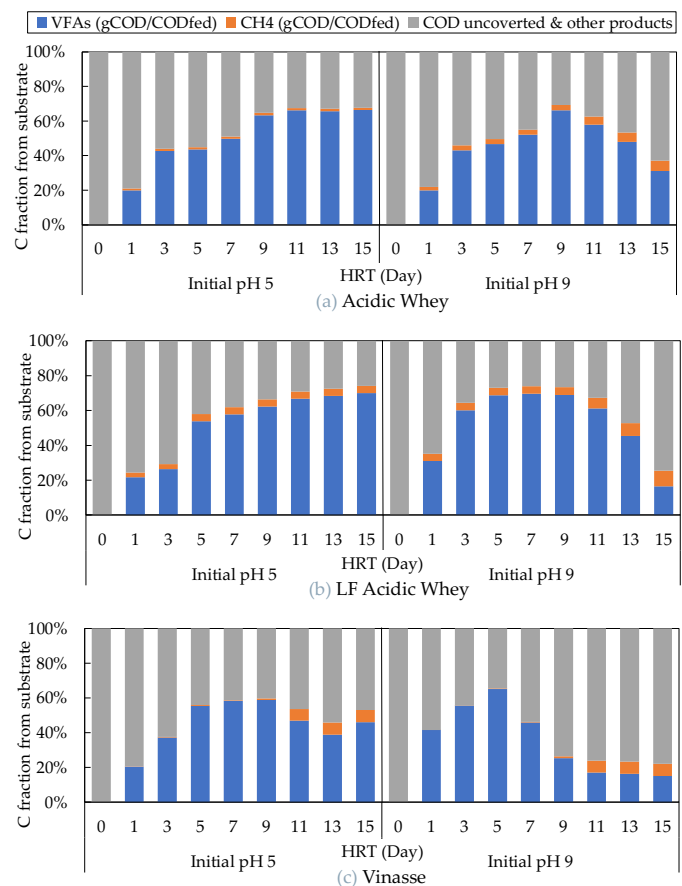


Figure 3: Carbon mass balance for the batch trials.

Overall, LF acidic whey at initial pH 5 displayed the best performance throughout the batch trial, despite having a relevant proportion of cumulated biomethane (2.65-9.06%). Under these conditions (Figure 3b, on the left) the peak share of VFAs was 70% for 15 days of HRT, with 4% of biomethane and 26% of residual carbon. Lastly, VFA production from this substrate experienced a net jump from day 3 (26%) to day 5 (54%), resulting in promising VFA accumulation within an HRT of 3-5 days.

3.2. Anaerobic Sequencing Batch Tests

Two AnSBRs were operated for 41 days with LF acidic whey under acidic environments to highlight the impact of the transition from short-term batch to long-term semi-continuous operation, under the effect of three different organic loading rates (OLR) i.e., 2.80, 4.67, and 6.53 g COD/L/d (food-to-microorganism F/M ratio of 0.3, 0.5, and 0.7 g COD/g VS, respectively). Figure 4 displays the VFA production and composition, together with the VFA yields.

3.2.1. Effect of OLR on VFA production and composition

The maximum VFA concentration was 15.47 g COD-VFA/L achieved with an ORL of 6.53 g COD/L/d on the last day of the experiments. During the operation, the average value was 8.71 ± 4.62 g COD-VFA/L. The average VFA yield was 0.50 g COD-VFA /g CODt and reached a peak of 0.66 g COD-VFA /g CODt on day 41st day. ORL had an impact on both total VFA production and yield. Applying an OLR of 2.80 g COD/L/d resulted in a maximum of 5.92 g COD-VFA /L and 0.57 g COD-VFA/g CODt for the two parameters, respectively. By contrast, higher values of 10.72 g COD-VFA /L and 0.64 g COD-VFA /g CODt were achieved when the two AnSBRs were operated with a higher OLR of 4.67 gCOD/L/d. In addition, the findings highlight the occurrence of a lag phase on days 15 and 29, needed for the microbial community to adapt to the change in the feed concentration from 9.8 to 16.3 and from 16.3 to 22.8 g COD/L, respectively.

The VFA profile obtained was mainly characterized by butyric acid. It comprised on average $39 \pm 13\%$ of the total VFA produced and had a peak concentration of 6.75 g COD/L (on day 36). Nonetheless, the VFA composition was characterised by an almost equal distribution of acetic ($24 \pm 10\%$), valeric acid ($14 \pm 5\%$), and propionic ($12 \pm 7\%$) with the highest concentrations of 3.54, 2.51, and 1.99 g COD/L, respectively. Interestingly, butyrate production was enhanced at higher feed concentrations, undergoing a two-fold increase from day 14 (2.15 g COD/L) till day 19 (5.65 g COD/L). During the first two weeks of operation (i.e., F/M=0.3) propionic acid concentration decreased from 1.44 gCOD/L (day 5) down to 0.39 gCOD/L (day 14), whilst caproic acid followed the opposite trend. This suggests a

possible consumption of propionate in favour of chain elongation. Caproate production almost tripled from day 29 (0.86 g COD/L) to day 41 (2.60 g COD/L), with an OLR of 4.62 g COD/L/d.

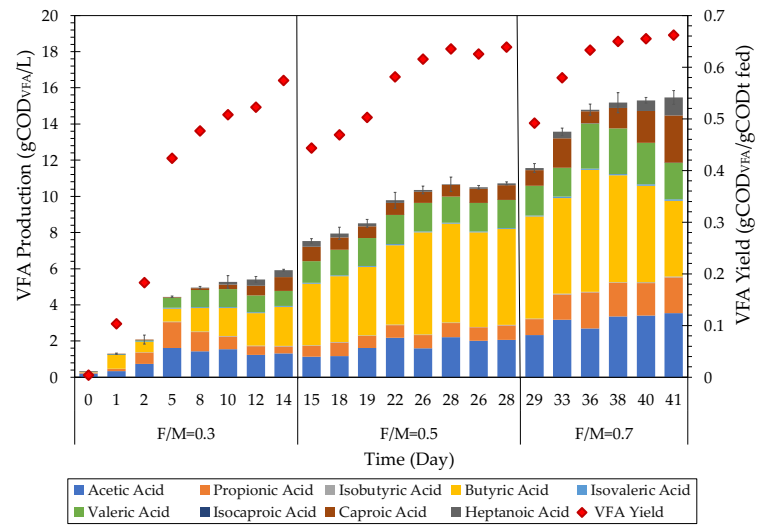


Figure 4: AnSBR results.

These results suggest a slight influence of OLR on the VFA profile, due to various prevailing metabolic pathways. Calero et al. demonstrated that lower OLRs enhance propionic and valeric acids, whereas higher values stimulated acetate, butyrate, and caproate. Likewise, Lagoa-Costa et al. reported analogous VFA profiles with HRT of 1-3 days, pH of 5, and ORL of 6 g COD/L [7]. In their study, the maximum butyrate production was detected operating the AnSBR at the highest feed concentration (up to 18 g COD/L, HRT = 3d), whilst applying lower values (6 and 12 g COD/L) resulted in a smaller concentration of such acid [7].

Table 4 collects the comparative data on VFA production, efficiency, and composition with cheese whey in AnSBR setups. The reported operating parameters were proven to achieve the best performances among the different ones tested. Overall, the results achieved in this long-term semi-continuous operation were comparable with the batch trial, proving AnSBR setups to be as suitable and capable of maintaining process stability as smaller bioreactors.

V (L)	T (°C)	pH	HRT (d)	OLR (gCOD/L/d)	Yield	VFA profile	Ref.
2	30	5	2	6	0.75 gCOD _{VFA} /g _{lactose}	HBu > HAc	[9]
2	30	5	2	6.5	0.87 gCOD _{VFA} /g _{lactose}	HBu > HAc	[7]
2	35	5	3.5	6.53	0.66 gCOD _{VFA} /gCOD _t	HBu > HAc > HCa	This study

Table 4: Comparative results for AnSBR trials.

4. Conclusion

Microbial mixed culture (MMC) tailored-anaerobic digestion (AD) for bio-based VFA production faces challenges like low yields and scalability. This project targeted increasing efficiency by filling the existing literature gaps on transitioning from optimised batch to semi-continuous setups. The acidogenic potential of regular acidic whey, lactose-free (LF) acidic whey, and vinasse was explored. These industrial wastes were first tested in an anaerobic batch setup for 15 days to highlight the effects of pH, hydraulic retention time (HRT), and substrate on the final VFA mixture. LF acidic whey acidogenic fermentation was then transitioned to an AnSBR configuration under pH 5 with different organic loading rates (OLRs).

As for the anaerobic batch trials, it can be concluded that regular acidic whey and LF acidic whey were more promising for bio-based VFA than vinasse. Regular acidic whey's slightly poorer outcomes were linked with lactose disaccharide, more demanding to digest. The initial acidic/alkaline environment combined with HRT, markedly influenced the total VFA production. Acidic conditions and longer HRT led to enhanced results for both the dairy residual streams, whilst an alkaline environment and shorter HRT boosted vinasse's performances. The composition of the feedstock (i.e., the substrate type), on the other hand, significantly determined the VFA profile of the final mixture. Acetic, propionic, butyric, valeric, and caproic acids were dominant, suggesting mixed-acid fermentation as the prevailing metabolic pathway. Interestingly, in the LF-acidic whey profile, butyric acid comprised 35–64% of the total VFA composition. Overall, the highest VFA concentration was 11.677 g COD-VFA/L with a corresponding VFA yield of 0.70 g COD-VFA/g COD_t fed achieved with LF acidic whey at an initial pH of 5 and HRT of 15 days.

This optimum was transitioned to an AnSBR setup, fixing the HRT to 3.5 days and controlling the pH to 5. Under long-term semi-continuous operating conditions, the performances of LF acidic whey were comparable with the results of batch acidogenic fermentation, accounting for maximum VFA concentration and yield of 15.7 g COD-VFA/L and 0.66 g COD-VFA /g COD_t, respectively. The VFA profile was mainly characterized by butyric acid (39% of the total VFA produced). ORL influenced both VFA production and composition. Higher values (4.67 and 6.53 g COD/L), despite

causing an initial lag phase for the microbial community, resulted in higher yields and stimulated butyric acid production. Bioreactor configuration did not influence the final VFA profile, proving AnSBR to be just as suited as smaller closed systems for process stability for longer operations.

To conclude, the upscaling strategy of acidogenic fermentation from a batch short-term setup to a long-term AnSBR reactor is promising for the bio-based production of VFAs from LF acidic whey under acidic environments. The semi-continuous configuration achieved comparable performance with shorter HRT (3.5 days) than batch trials (15 days), bearing proof of MMC tailored-AD scalability for bio-based VFAs.

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